LEAF SENESCENCE IN TWO POTTED CHRYSANTHEMUM (*DENDRANTHEMA GRANDIFLORA* RAMAT) CULTIVARS TARA AND BOALDI

By

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A man will occasionally stumble across the truth but most of the time he will pick himself up and continue on.

Winston Churchill

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The effect of storage duration and temperature, cultivar, fertilizer level, and type of bud removal were tested in a series of experiments to characterize leaf yellowing in potted chrysanthemums and to elucidate the possible stresses that may induce premature leaf senescence. Boaldi leaves became brittle and easy to break under high nutritional levels, but they never developed any visual chlorosis regardless of the treatments imposed. Tara was very susceptible to prolonged dark storage (6 days at 21°C) when plants were produced with the highest fertilizer level (300 mg $N.l^{-1}$, from 20N-4.7P-16.6K), and after storage leaves were chlorotic and wilted within a week in the interior holding rooms. Multiflowered Tara plants, in which apical buds were removed and lateral buds were allowed to flower showed a distinctive pattern of veinal clorosis, while disbudded

plants, in which all laterals buds were removed, did not. Tara was very susceptible to storage at 12°C when grown in late spring or early fall regardless of the fertilizer rate and type of bud removal. Symptoms similar to chilling injury i.e. loss of turgor and wilting were observed when the plants were unboxed or within the next few days. No symptoms of chlorosis or chilling injury were observed in experiments conducted in winter.

Dark-induced senescence of detached leaves showed that chlorophyll and total soluble proteins steadily decreased, and proteolytic activity increased in Tara during a 12-day period. In contrast, Boaldi leaves maintained the initial high chlorophyll and protein levels and did not show a significant increase in proteolytic activity. Attached and detached leaves exhibited similar senescence patterns for each cultivar. Ethylene ($100\mu l.l^{-1}$) accelerated the rate of chlorophyll loss in detached Tara leaves but had no effect on Boaldi. These results suggest that Boaldi is a type of 'stay-green' or non-yellowing cultivar, in which genes involved in the initiation of senescence have been altered.

CHAPTER 1
INTRODUCTION

Flowering potted chrysanthemum *Dendranthema* x *grandiflora* (Ramat) Kitamura, is one of the most popular ornamental plant in the U.S. (Barletta, 1996). However, space for potted florist chrysanthemum was 7% lower in 1996 compared to 1995 (United States Department of Agriculture, 1997). Introduction of other potted flowering plants is increasing rapidly and competition is becoming stronger. One of the key factors in this competition will be quality of potted crops (Noordegraaf, 1995). Longevity and interior performance of flowering potted plants is a primary area of concern for commercial producers, floral buyers and consumers (Nell and Hoyer, 1995). In a market survey by Shafer and Kelly (1986), results indicated that a consumer's interest in longevity would take precedence over price.

Quality of flowering potted plants involves the interior performance and continued development in the home or office of the entire plant. Premature senescence of leaves, bracts or flowers can degrade the interior performance of flowering potted plants (Nell and Høyer, 1995). Leaf chlorosis and necrosis are common postharvest problems of some potted chrysanthemum cultivars following shipping under dark conditions, which

severely reduces postproduction quality (Carver et al., 1993; Nell et al., 1989; Takinagawa et al., 1995). Postproduction longevity of potted flowering plants depends on the species and cultivar as well as on the production environment, storage and interior conditions. Previous research emphasized the effect of production and postproduction factors on flower longevity (Nell et al., 1989; Roude et al., 1991); however, few studies have addressed the leaf postproduction performance of potted chrysanthemums.

These studies were conducted to elucidate the production factors and storage conditions that may trigger premature leaf senescence and to characterize dark-induced leaf senescence patterns in Tara and Boaldi potted chrysanthemums.

CHAPTER 2
LITERATURE REVIEW

Introduction

Potted flowering crops are subjected to several days of dark storage and transport before they reach the market. Prolonged dark storage and adverse temperatures often accelerate senescence processes, which may result in the complete loss of horticulture commodities (Huber, 1987).

Most basic research on leaf senescence involved studies of agronomic crops in which leaf area index and leaf area duration are predominant determinants of yield. Lipton (1987) pointed out the lack of studies on senescence of leafy vegetables, a group of crops of major economic importance. Similarly, senescence of potted flowering plants has attracted only limited attention. Nevertheless, as Lipton (1987) suggested, the results obtained with leaves of oats, rice, soybean and tobacco must be considered to help elucidate the senescence mechanisms and the possible factors involved in premature leaf senescence.

Leaf Senescence

Leaf senescence is the sequence of biochemical and physiological events leading to cellular disassembly and the mobilization of released material that eventually results in the death of the organ (Thomas and Stoddart, 1980). The physiological advantage of this recycling process has led to viewing senescence as an endogenously controlled developmental process rather than a passive degeneration(Thomas and Stoddart, 1980). It is generally accepted that senescence is regulated by differential gene expression, including activation of new genes (Smart, 1994).

The most obvious symptom of leaf senescence is yellowing, which is due to the preferential degradation of chlorophyll (Thimann, 1980). Plants destroy chlorophyll and other porphyrines because the senescent cells must be detoxified of dangerous photodynamic molecules, which are liberated when the corresponding apoproteins are mobilized. Detoxification is necessary because orderly metabolism can take place only if the senescent cells are intact and compartmentalized (Matile et al., 1989).

Chloroplasts are one of the earliest sites of catabolism in leaf senescence. Chloroplasts store 70-80% of the nitrogen in mature leaves (Thomas and Stoddart, 1980). Sabater et al. (1990b) showed that senescent chloroplasts have significant NADH

dehydrogenase activity. Their results suggested that chloroplasts become a respiratory organelle in the dark. Accumulation of osmiophilic globular bodies in senescing plastids indicates a build-up of lipid material originating from thylakoid breakdown (Thomas and Stoddart, 1980). Ribosomes are lost from the cytoplasm and from the stroma of the chloroplasts (Brady, 1988). As senescence advances, plasmalemma integrity is lost, and relatively late changes occur in mitochondrial and tonoplast membranes (Thompson, 1988). Mitochondrial function continues until quite late in senescence, and that may be related to a continued need for energy by the activated process of senescence (Solomos, 1988).

Loss of membrane integrity is initiated during the early stages of senescence (Thompson, 1988). Membranes are highly prone to free radical attack since unsaturated fatty acids are major components of most membrane lipid bilayers (McKersie et al., 1988). The consequences of free radical attack on membranes include the induction of lipid peroxidation, lysis and fatty acid deesterification. During senescence there is a strong relationship between free radical and membrane rigidification (McKersie et al., 1988).

Biochemical studies have revealed a large number of metabolic changes during leaf senescence: protein and nucleic acid levels decline during senescence (Peoples and Dalling, 1988), but the selective activation of the synthesis of certain

mRNAs and proteins seems to mark the initiation of the active processes leading to senescence (Brady, 1988; Lohman et al., 1994). There is a marked increase in proteolytic enzymes (Martin and Thimann, 1972; Thimann, 1980), nucleases (Blank and McKeon, 1991) and enzymes of chlorophyll catabolism (Thomas et al., 1989).

Internal Factors Affecting Senescence

Redirection of nutrients from leaves to flowers and fruits was the first theory of leaf senescence (Molisch, 1938). The diversion of nutrients away from vegetative parts and to the developing fruits has been considered to play a causal role in monocarpic senescence (Noodén, 1980). In monocarpic species, leaf senescence is delayed by removal of immature fruits (Noodén, 1980). The number of leaves that senesced during the reproductive phase was related to the number of fruits left to mature on the plant (Crafts-Brandner and Egli, 1987). This relationship suggests that changes in the ratio of photosynthetic source size to reproductive sink size may influence the course of monocarpic senescence. Reproduction certainly seems to exert a strong influence on the timing of leaf senescence in annual crops.

Policarpic plants, on the other hand, decline much more slowly than monocarpic plants, and longevity is characteristic of the species (Noodén, 1988b). This suggests genetic determination of life span, but longevity of policarpic plants could also be

determined more by increased vulnerability to stress than by a distinctive internally programmed degeneration (Noodén, 1988b).

There is considerable indirect evidence supporting cytokinin involvement in delaying senescence in a large number of species (Kulaeva et al., 1996; Mok, 1994; Noodén et al., 1990; Singh et al., 1988; Thimann, 1980). Exogenous cytokinin treatments have delayed senescence in a variety of plants (Ben-Jacoov et al., 1985; Clark et al., 1991; Kao, 1980; Tjosvold et al., 1994). Other correlative evidence involved comparisons of endogenous cytokinins in young, maturing and senescing tissues; leaf senescence was generally found to be accompanied by a decrease in endogenous cytokinins (Beever and Woolhouse, 1974; Sitton et al., 1967). However, a study of cytokinins activity in 'Polaris' chrysanthemum showed similar amounts of cytokinins throughout development (Holland et al.,1981). They indicated that the relatively high level of cytokinins in the roots in 16- week-old chrysanthemum could be associated with the formation of bottom breaks observed after the senescence of the flowers and the perennial nature of the species.

It is widely accepted that cytokinins transported from the roots via the xylem sap delay leaf senescence (Noodén et al. 1990). In sorghum and rice, cytokinin contents in xylem sap were found to be higher in a high-yielding senescing cultivar with delayed leaf senescence than in an early-senescing cultivar (Ambler et al., 1992; Soejima et al., 1995).

Treatments which decrease cytokinin production by the roots such as salt stress, hypoxia or mineral deficiency, promote leaf senescence possibly by reducing the cytokinin level in the leaves (Kuiper et al., 1989; Wagner and Beck, 1993). On the other hand, treatments that delay leaf senescence, such as bud removal and decapitation of tomato and tobacco plants resulted in an increase in the concentration and the total flux of cytokinins in bleeding xylem sap (Colbert and Beever, 1981). They suggested that the delay of leaf senescence observed in disbudded plants was due not only to a decreased demand in the shoot, but also to an increased supply from the roots. Van Staden and Carmi (1982) found similar results in decapitated and partially defoliated *Phaseolus vulgaris* and speculated that the increase in the root to shoot ratio resulting from partial excision of shoot organs may enhance the cytokinin supply from the roots to the rest of the shoot. In a later work, Carmi and Van Staden (1983) demonstrated that partial root excision of decapitated and partially defoliated plants reduced the accumulation of cytokinins in the leaves.

Cytokinins delay senescence by stimulating synthesis of chloroplastic DNA and photosynthetic enzymes, as well as grana formation and chloroplast replication (Van Staden et al., 1988). Sabater et al. (1990a) showed that cytokinin increases the levels of chloroplast mRNAs usually expressed during the earliest stages of chloroplast development and prevents the translation of mRNAs senescence polypeptides.

Protein and nucleic acid levels decline during senescence (Brady, 1988), although this decline seems to affect only certain proteins, while others are unaffected or increase. Among the proteins with higher activity during senescence are proteolytic enzymes. Cytokinins prevent this increase in proteolytic activity and decrease lipase and lipoxygenase, which are involved in membrane breakdown (Van Staden et al., 1988). The effect of cytokinins in membrane integrity may be important in delaying senescence, since it maintains compartmentation and prevents leakage of nutrients and enzymes.

Recent research on mutants and transformants has provided the most convincing evidence linking endogenous cytokinins to plant development. Expression of the *tmr* gene of *Agrobacterium* in plants has led to alterations in developmental patterns (decrease in apical dominance, delay in senescence and increase in chlorophyll formation) with accompanying increases in endogenous cytokinins (Mok, 1994).

In some systems, auxins and gibberellins have been reported to influence leaf senescence (Thimann, 1980). Gibberelic acid retarded loss of chlorophyll in *Hedera-Helix* L. (Horton and Bourguoin, 1992), and *Alstroemeria pelegrina* L. (Dai and Paull, 1991; Jordi et al., 1993; van Doorn et al., 1992). In *Alstroemeria*, Jordi et al. (1994) found no evidence that GA_3 delays the loss of chlorophyll by affecting the transport of nutrients within the cut flowers. Ethylene has the opposite

effect to cytokinins as it accelerated many of the physiological changes normally associated with senescence, and abscisic acid enhanced the rate of chlorophyll loss (Gepstein and Thimann, 1981; Mattoo and Aharoni, 1988). Recent work with transgenic or mutant plants has provided direct evidence for the role of ethylene in leaf senescence (Grbić and Bleeker, 1993; Picton, 1993). Ethylene hastens the progression of senescence by activating senescence-associated genes, while repressing photosynthesis-associated genes (Grbić and Bleeker, 1995).

Abscisic acid (ABA) accelerates the senescence of leaf discs of a variety of species but is less effective when applied to attached leaves (Thomas and Stoddart, 1980). The levels of ABA increase under conditions of stress such as drought, low temperatures and salinity (Baker and Lachno, 1989; Pell and Dann, 1991), which predispose the plant to senescence.

The flux and quality of light reaching leaves at the bottom of a canopy is very different from that of the incident light. Light fluxes decrease exponentially as light penetrates the canopy and leaves absorb red (R) light while transmitting far-red (FR) light. Guiamét et al. (1989) showed that intracanopy changes in R:FR are important in the modulation of progressive senescence in natural environments. There is plenty of evidence supporting the involvement of phytochrome as the receptor controlling leaf senescence in response to light (Behera and Biswal, 1990; Biswal and Biswal, 1984; Casal and Aphalo, 1989; Tucker, 1981).

In an attempt to simplify the study of senescence, model systems have often been used, such as detached leaves or segments, and dark treatment, to induce senescence. Smart (1994) emphasized the dangers in assuming that this artificial system represents a true picture of natural senescence in the plants. In fact, Becker and Apel (1993) found that only a minor part of the mRNA changes observed during dark incubation of detached leaves was related to leaf senescence, whereas stress-related transcripts appeared to predominate quantitatively. Nevertheless, model systems are justified in certain circumstances, such as comparisons of varieties differing in their patterns of senescence under natural conditions and subject to the same treatment (Smart, 1994).

Genetic Variability in Leaf Senescence

Genetic variation exists for symptoms of leaf senescence. Genotypes with leaves which remain green for longer than normal have been termed stay-green or non-yellowing varieties (Smart, 1994). Examples of delayed or inoperative senescence have been found in maize (Crafts-Brandner et al., 1984), sorghum (Duncan et al., 1981), *Phaseolus* (Ronning et al., 1991), rice (Mondal and Choudhuri, 1985), wheat (Boyd and Walker, 1972), fescue (Thomas and Stoddart, 1975), soybean (Guiamét et al., 1991), and several fruits and trees.

Smart (1994) emphasized that the stay-green behavior in one genetic line may have only a superficial resemblance to the character in another and may arise from quite different underlying physiological and biochemical modifications. In most cases of delayed senescence there have been few biochemical studies of the character. During senescence, some of these mutants retained both chlorophyll and photosynthetic competence ('functional' *stay-greens*) while others kept their chlorophyll but degraded other components of the photosynthetic apparatus ('non-functional' *stay-greens*) (Thomas and Smart, 1993).

Functional types of stay-green may arise after alteration of genes involved in the timing of the initiation of senescence and the regulation of its rate of progress. Since these stay-green types continue to photosynthesize longer than normal, higher yield in crops for which carbohydrate is a major component of the harvest is expected.

In contrast, non-functional stay-green types look green but lack photosynthetic competence. These types of stay-green plants may arise by alteration of genes which regulate chlorophyll metabolism (Thomas and Smart, 1993). In maize both stay-green types exists: in a stay-green variety (FS854) studied in detailed, the levels of chlorophyll and PEP carboxylase started to decline on schedule but the rate of decrease was reduced compared to other varieties (Crafts-Brandner et al., 1984), which suggested that FS854 is a functional stay-green type. In another

maize line, chlorophyll was retained while the level of PEP carboxylase declined as usual, indicating a non-functional stay-green type (Thomas and Smart, 1993).

A mutant genotype of *Festuca pratensis* L. is characterized by a complete disabling of the leaf yellowing process, but other aspects of senescence, such as soluble protein degradation proceed according to schedule (Thomas, 1977; Hilditch et al., 1989), which denotes that the mutant is a non-functional stay-green. Inheritance studies in *Festuca* have established that the stay-green phenotype is the result of a mutation at a single nuclear locus, designated *Sid* (senescence-induced degradation) (Thomas, 1985). The first step in the pathway of chlorophyll breakdown, removal of the phytol side-chain, occurs normally in the mutant and this reaction takes place while chlorophyll is still attached to its binding protein in photosynthetic membranes. The next steps do not function in mutant leaf tissue (Thomas et al., 1989). The associated membrane proteins, which are normally degraded at the same time as chlorophyll during senescence, are retained in the mutant so that senescent tissue maintains significant amounts of protein nitrogen in the form of pigment proteolipids (Hilditch, 1986; Hilditch et al., 1989).

Stress and Senescence

Many natural and anthropogenic stresses induce or accelerate changes that resemble the senescence syndrome. The processes

leading up to cell death have been grouped into three categories: 1) programmed cell death; 2) necrosis, which is due to massive trauma such as acute chilling injury, wounding or reaction to microbia pathogens, and 3) chronic degeneration, visualized as an accumulation of sublethal damage with time. The main characteristic of cell death, whether from senescence, acute stress, or aging, seems to be the loss of the cell's ability to maintain homeostasis. The cell is alive as long as it can maintain its entropy below that of its environment; this requires not only integrity of its membranes but a constant input of energy (Noodén, 1988a). Once the cost of self-maintenance, i.e. basal metabolic rate, repaired mechanism, etc., can no longer be met, the cell dies (Penning de Vries, 1975).

Noodén (1988a) emphasized the distinction between acute stress (short-term, high intensity) and chronic (long-term, low intensity). While extreme drought (acute stress) simply overwhelms and quickly kills a plant or organ, chronic, sublethal drought may accelerate senescence. Many stresses induce protein degradation, an important feature of senescence (Rabe, 1990). Water stress affects many processes in the same way as senescence, i.e. photosynthesis, protein synthesis, chlorophyll and protein levels declined (Levitt, 1980b). Water stress also stimulated stomatal closure and increased concentration of ABA (Levitt, 1980b). Increases in ABA have been correlated with increases in ethylene, which is known to accelerate senescence

processes (Mattoo and Aharoni, 1988). Many stresses, including mechanical wounding, ozone, chilling, flooding, and drought induce ethylene and associated premature senescence (Yang and Hoffmann, 1984).

It is well documented that hormones such as ABA also act as root-to-shoot signals during water stress (Davies and Zhang, 1991; Shashidhar et. al., 1996). Recent evidence indicates that root cytokinins may also contribute to the signal system between root and shoot (Cizkova, 1992; Goicoechea et al., 1996; Kuiper et al., 1989; Shashidhar et al., 1996). Chapin (1991a) postulated that plants respond to environmental stresses in basically the same way: through a decline in growth rate and in the rate of acquisition of all resources. Plants exhibit a centralized system of stress response that can be triggered by a diverse range of stresses. Chapin (1991a) proposed that this centralized system is hormonally mediated but involves integrated changes in nutrient, water, carbon, and hormonal balances of plants.

The effect of stress on the lifespan of the plant may differ depending on the plant's developmental stage at the time the stress occurs (Pell and Dann, 1991). Longevity of potted plants can be affected by numerous stresses arising during the production of the crop as well as during transportation.

Senescence of potted plants is cultivar and/or clone dependent. Different *Ficus benjamina* clones have different longevities under interior conditions (Ottosen and Høyer, 1988).

Poinsettia cultivars also have different levels of leaf drop and bract abscission (Scott et al., 1982; Nell and Barrett, 1986b) and chrysanthemum cultivars have different interior longevities (Nell et al., 1990). However, the final quality and longevity of potted flowering crops when they reach the consumer is the result of an interaction of variety selection, environmental factors, cultural practices and postproduction handling conditions (Nell, 1991).

Temperature, irradiance, and fertilization during production have been manipulated to grow plants more tolerant to prolonged storage (Miller et al., 1993, 1995; Nell et al., 1989). With the introduction of new cultivars, evaluation of the storage conditions that will optimize postproduction performance is an ongoing process (Tijskens et al. 1996).

Production Factors and Plant Longevity

Irradiance

Foliage plants have been successfully acclimatized to prolonged storage conditions and to survive low light interior environments by producing them under moderate to low irradiance (Conover and Poole, 1984). However, it has generally been shown that flowering crops require high irradiance levels during production. 'Bright Golden Anne' potted chrysanthemum had the

greatest dry matter when grown with high photosynthetic flux (PPF, 1.8-21.6 $mol.d^{-1}.m^{-2}$) (Karlson and Heins, 1992). Heaviest plants produced the highest flower/plant weight ratio, which were also the ones with most rapid rates of development (Cockshull and Hughes, 1967).

In 'Iridon' and 'Mountain Peak' potted chrysanthemum, days-to-flower decreased and inflorescence diameter, total chlorophyll, and interior plant longevity increased when plants were maintained at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ compared to plants shifted to 300 or 100 ($\mu mol \cdot m^{-2} \cdot s^{-1}$) 8 weeks after planting (Nell et al., 1990).

Begonia x cheimantha produced at low light levels showed decreased postharvest performance, with flower abscission negatively correlated with plant sucrose and starch concentrations (Fjeld, 1986, 1992). The postharvest quality of 'Enchantment' lily is greatest when plants were grown during the high-light periods of the year (Swart, 1980).

Increased flower longevity has been associated with carbohydrate reserves in cut (Marissen and La Brijn, 1995; Paulin 1986) and potted flowers (Monteiro, 1991). Leaf blackening in *Protea* (Bieliski et al., 1992; Dai and Paull, 1995; McConchie and Lang, 1993), leaf chlorosis in Easter lilies (Jiao et al., 1986; Miller et al., 1995), and inflorescence and stem necrosis in *Vitis vinifera* L. (Keller and Koblet, 1994) have been related to reduced stored carbohydrates. However, increasing the leaf

carbohydrate pool by raising CO_2 levels did not reduce postproduction leaf chlorosis in miniature roses (Rajapakse et al., 1994).

In poinsettia, carbohydrate-depleting treatments (low irradiance, leaf shading, leaf removal, high night temperature) increased cyathia abscission (Miller and Heins, 1986; Moe et al., 1992), but 'Amy' poinsettias conditioned by 1 to 3 weeks of low light before anthesis exhibited less leaf abscission after 30 days in an interior environment (Nell et al., 1990).

Leaf chlorosis was first documented in chrysanthemum cut flowers in the 60s (Woltz, 1969) and was associated with the accumulation of excess photosynthate (Woltz and Engelhard, 1971). A decline in photosynthesis and premature senescence occurred when carbohydrates built up in leaves of wheat and *Medicago* (Araus and Tapia, 1987; McKersie et al., 1992). However, in chrysanthemum, developing flowers are major sinks and become the only site for the net accumulation of dry matter (Cocksull and Hughes, 1968) so that excess photosynthate in the leaves is not expected. Furthermore, in potted chrysanthemum, carbohydrates have been found to preferentially accumulate in shoots and roots (Monteiro, 1991). Moreover, holding chrysanthemum cut flowers in a sucrose solution following gamma irradiation (quarantine purposes) delayed wilting and foliage yellowing (Hayasi and Todoriki, 1996).

Temperature

It has been suggested that the time of anthesis of many cultivars of chrysanthemum is regulated by the 24-h mean temperature and not by either the day or night temperatures alone (Cockshull et al., 1982; Pearson et al., 1993). This relationship has been shown to be generally true for 24 h mean temperatures in the range 10-20C, but it did not hold at mean temperatures above 22C because anthesis was delayed (Whealy et al., 1987). Anthesis was delayed if either day or night temperatures exceeded 26C (Karlson et al., 1989).

The principal method to control flowering on chrysanthemum is daylength and, as a short-day plant (Post, 1950), opaque covers must be drawn over the crop in the afternoon in summer for at least 12 h for rapid flowering. Greenhouse temperature is highest in summer and the temperature under the black cover frequently exceeds 25C. Cockshull and Kofranek (1994) reported that high night temperatures at the onset of floret initiation delayed anthesis, and young expanding leaves turned yellow (assessments were made at anthesis), although older leaves remained dark green. The longer the duration of the exposure at high night temperatures, the greater the number of leaves affected. The authors suggested that leaf chlorosis was a response to the magnitude and sign of the difference between

day and night temperature, rather than to high night temperature per se.

Control of stem elongation can be achieved by manipulating the difference between day and night temperature (DIF). A reduction of stem elongation (highly desirable in potted plants) can be obtained by growing plants under higher night than day temperatures (Erwin et al., 1989). This phenomenon has been termed "negative DIF" and has been adopted in many commercial greenhouses. However, negative DIF reduced percentage dry matter in *Lilium longiflorum* Thunb. (Zieslin and Tsujita, 1988), total leaf carbohydrates in poinsettia (Senecal et al., 1989) and increased postharvest cyathia abscission in certain poinsettia cultivars (Moe et al., 1992).

In Easter lilies, negative DIF induced foliar chlorosis (Erwin et al., 1989) and significantly reduced leaf and stem total carbohydrate content (Miller et al.,1993). Miller et al. (1993) pointed out that rapid respiration of accumulated leaf sugar in the early hours of the warm night resulted in the concomitant reduction in carbohydrate concentration compared to plants grown under a positive DIF regime.

Nutrition

Extensive work has shown that cut (Kofranek, 1980) and potted chrysanthemums (Crater, 1980) requirements for fertilizer are more critical during the first half of the growing season.

Lunt and Kofranek (1958) emphasized that maintaining high N levels early in the growth of chrysanthemums was essential since deficiencies suffered during this stage of growth could not be overcome by later N fertilization. Nitrogen accumulation rate (mg N/day) in chrysanthemum plants peaked by the 6th week of growth and then decreased (Elliott and Nelson, 1983; Woodson and Boodley, 1983). Since fertilizer was applied throughout production, the decrease of NO_3^- from the vegetative tissues indicated a decline in the plant capacity to accumulate NO_3^- during inflorescence development (Woodson et al., 1984).

In chrysanthemums, high nitrogen rates produced very thick, crinkled, and brittle leaves (Lunt and Kofranek, 1958), and more succulent foliage (Waters, 1965; Wesemberg and Beck, 1964). Keeping quality of foliage (Braswell et al., 1982; Joiner et al., 1983; Poole and Chase, 1987) and several flowering plants (Carver et al., 1993; Nell and Barrett, 1990; Serek, 1990; ter Hell and Hendricks, 1995) improved when moderate levels of fertilizer were applied during production. High and sustained levels of fertilizer until flowering reduced flower longevity and leaf quality in chrysanthemums (Carver et al., 1993; Ishida et al., 1983; Joiner and Smith, 1961; Lunt and Kofranek, 1958; Nell et al., 1989; Roude et al., 1991).

It has been argued whether the adverse effect of sustained N on the keeping quality of ornamental plants is due to an increase in salts in the media and possible root damage (Carver et al.,

1993; ter Hell and Hendricks, 1995) or to a specific N effect on the plant. Ter Hell and Hendricks (1995) reported that increasing K in the media increased the electrical conductivity of the media (EC) more than N, but K had no effect on the keeping quality. Their results suggested a specific nitrogen effect.

Most or all plants do not regulate nitrogen uptake (Rabe, 1990). Evolution of land plants under conditions of nitrogen limitation resulted in little selection pressure for mechanisms regulating nitrogen uptake or reduction (Miflin and Lea, 1980). Rabe (1990) pointed out the dangers of applying excess nitrogen in modern agriculture to 'stimulate' growth, probably to the point where the anabolic mechanisms of the plant can not cope with it. High N in soils increases K+ uptake and decreases Ca^{++} and Mg^{++} uptake, increasing the $K:(Ca^{++} + Mg^{++})$ ratio (Mengel and Kirby, 1982). Wheat pastures with a high $K:(Ca^{++} + Mg^{++})$ ratio might cause grass tetany and frothy bloat in wheat forage (Fernandes and Rossiello, 1995).

Salinity, whether natural or induced by irrigation and heavy fertilization, is a problem of increasing importance in agriculture because almost all crop plants are more or less salt sensitive (Kramer, 1983). Photosynthetic capacity at high PPF (photosynthetic rate at saturation PPF) decreased in tomato grown in nutrient solution with high EC(4.5 $mS.cm^{-1}$)(Xu et al., 1994) and in strawberry grown at high NaCl (8.6 $mS.cm^{-1}$) (Awang and Atherton, 1994). The photosynthetic depression caused by high EC

was attributed more to a reduction in stomatal conductance than to mesophyll conductance (Xu et al., 1994). Stomatal and cuticular transpiration were decreased by high EC, and leaf wax content increased (Xu et al., 1995), which suggested that increased wax deposition prevents water loss from the cuticle in tomato leaves. High EC affects plants physiology through decreasing water uptake (Ehret and Ho, 1986). Even under sufficient water supply, substrate salinity can induce water stress, especially when evapotranspiration demand is high (Awang and Atherton, 1994; Zeroni, 1988).

Salinity reduced growth and yield of plants (Greenway and Munns, 1980). A mixture of ions is less damaging to plants than a single salt treatment (Kofranek et al., 1952; Hughes and Hanan, 1978). Some symptoms included stunting and succulent leaves (Handley and Jennings, 1977; Longstreth and Nobel, 1979), necrotic margins (Oertli, 1966) and premature senescence of leaves even in osmotically adjusted plants (Prisco and O'Leary, 1972).

The adverse effects of low external osmotic potential is often remedied by uptake of inorganic solutes to maintain cell turgor (Flores et al., 1977). However, this accumulation of ions can produce problems of mineral toxicity and nutritional imbalance. Both inorganic and organic solutes contributed to osmotic adjustment, but the way in which the osmotic adjustment

is achieved will determine at least partially the salt tolerance of a species or genotype (Richardson and McCree, 1985).

Osmotic adjustment based on the synthesis of organic solutes requires an important energy expenditure (Alarcon et al., 1994; Munns, 1993), while accumulation of ions in the vacuole provides energetically 'cheap' solutes for osmotic adjustment (Flores et al., 1977). However, if ion uptake is to be used for turgor maintenance, an effective regulation of ion concentration in the shoots is required. Because the respiratory cost of maintenance is a large fraction of the total carbon budget of plants, any variations in maintenance requirements resulted in significant alterations in productivity (Amthor, 1984; Lambers et al., 1983). Penning De Vries (1975) pointed out that salinity can increase the cost of maintaining intracellular ion concentration markedly.

An increase of the plant salt concentration generally stimulates processes for maintenance of ion concentration (Watson, 1970). Schwarz and Gale (1981) reported that the increased use of assimilates for maintenance of *X. strumarium* grown in a culture solution of -0.5 MPa was responsible for about 24% of the reduction in dry weight growth. At relatively high night temperatures the maintenance cost was large and the rate of biosynthetic processes was stimulated, so that the pool of reserve carbohydrates was depleted before the end of the night (Schwarz and Gale, 1983).

On the other hand, ion uptake is one of the major sinks for respiratory energy in the roots (Lambers et al., 1983; van der Werf et al., 1988). At relatively high rates of growth and nitrate uptake, the contribution of ATP production for ion uptake accounts for 38% and 25% of the total ATP production for *Carex diandra* Schrank and *Carex acutiformis*, respectively (van der Werf et al., 1988).

Shipping Duration and Temperature

Transportation or storage in darkness results in plant stress whether the plants are well-watered or not. Metabolic changes during dark starvation has been studied extensively (Krapf and Jacobi, 1975; Postious and Jacobi, 1971). Woltering (1987) reported that the effect of dark storage on potted plants is similar to the effects of exogenous ethylene: plants showed abscission of flowers, bracts or leaves, and symptoms of leaf yellowing and browning.

The action of light in retarding senescence has been attributed to its role in photosynthetic production of organic nutrients (Elamrani et al., 1994; Satler and Thimann, 1983), although there is clear evidence of the role of phytochrome as the major photoreceptor involved in light-mediated senescence retardation (Biswal and Biswal, 1984).

Potted ornamental plants usually require several days to arrive at the final destination from production areas and are often exposed to temperature extremes unless the shipping environment is temperature controlled (Collins and Blessington, 1983). Optimum shipping temperatures vary considerably among genera and shipping duration (Poole and Conover, 1983; Sterling and Molenaar, 1986).

van Gorsel (1994) emphasized that the most important measure during long transport of floriculture commodities is temperature. Temperature is the most important environmental factor determining the rate of respiration (Hällgree and Öquist, 1990). With increases in temperature, there is an increased rate of reactions (Salisbury and Roos, 1992) and in the dark respiratory processes are affected. Low temperatures slow the depletion of carbohydrate reserves (Salisbury and Roos, 1992) and decrease ethylene production (Graves and Gladon, 1985).

Elevated temperatures during shipping or storage increase transpirational loss and reduce plant quality. Stomates of many species close in the dark but the degree of closing may vary among species (Rajapakse et al., 1988). Chrysanthemums stored in the dark at 25°C lost considerable amounts of water through transpiration (Rajapakse et al., 1989). Pre-treatment with ABA and silver thiosulphate (STS) reduced chrysanthemums transpiration rate during storage (Rajapakse et al., 1989).

In a study of twenty economically important potted plants subjected to six storage periods (1-21 days) and six constant temperatures (5-30°C), Sterling and Molenaar (1986) reported that a wide range of temperatures gave optimum storage (minimum quality loss) when the plants were stored for a relatively short time, but a progressively smaller range as the products were stored longer. All plants were sensitive to high temperature and most plants were sensitive to chilling temperatures.

Nell et al. (1989) found that storage duration had little effect on 'Mountain Peak' chrysanthemum at 4 and 16°C but decreased longevity 7 days when duration was extended from 2 to 7 days at 24C. Sterling and Molenaar (1986) indicated that the optimum shipping conditions for chrysanthemum hybrids was 1 day at 10C. Higher and lower temperatures were suboptimal.

The chilling sensitivity of the species defines the lower limit of the acceptable temperature range. Chill-sensitive plants, usually of tropical or subtropical origin, are injured by exposure to temperatures within the range 0-15°C (Abbott et al 1987; McMahon et al. 1994; Semeniuk et al. 1986). However, cultivar variability also exists (Hummel and Henny, 1986; Moline and Semeniuk, 1983).

At low temperature, the structure of cell membranes is modified. The membrane lipids become more rigid, which alter membrane permeability and enzyme activities (Côme, 1991). Response to the duration of exposure to chilling stress depends

upon the plant and tissue, but early symptoms of chilling-injury are reversible on re-warming, while severely chilled tissue deteriorates further when returned to warmth (Salveit and Morris, 1990). Symptoms of chilling injury were evident immediately after exposure in *Episcia* but required 27 h after chilling in *Dieffenbachia* (McMahon et al., 1994). Niki et al. (1979) suggested that irreversible changes in the tonoplast at chilling temperatures may govern the ability of the plant to survive.

The effect of prestorage conditioning to prevent chilling injury is well documented (Salveit and Morris, 1990). Early-season (September-October) lemons showed greater chilling injury than did mid-(January) or late-season (February-March) fruits (Houck et al., 1990). Prestorage condition of grapefruit at 16° C was better at minimizing chilling injury than 21 or 27 °C (Hatton and Cubbedge, 1983). Growth regulators and plant hormones have been used to modify chilling sensitivity (Abbott, et al., 1987; Semeniuk et al., 1986; Wijeratnam et al., 1995).

Summary

Premature leaf senescence in potted flowering crops can be induced by numerous stresses during crop production, storage and transport, and often an interaction of stresses occur. Environmental factors that reduce leaf carbohydrate status such as low irradiance, negative DIF, or high nutritional levels

result in plants less tolerant to prolonged dark storage. Also, production factors that limit root growth (high fertilizer rates, overwatering, salinity, etc) are associated with premature leaf senescence, probably through a decrease in cytokinin production and/or stimulation of ABA and ethylene production. Prolonged dark storage and high temperatures during shipping induce carbon starvation and leaf senescence. On the other hand, low temperatures applied during transport to slow senescence processes may damage chilling sensitive crops.

In a number of very important agronomic crops, stay-green varieties have higher yields, increase resistance to diseases and stress conditions, and possess leaves with higher nutritional quality. Interest in this important character in ornamental crops has been largely incidental. In floriculture crops, breeding programs have focused mainly on improving color, size, form, and longevity of the flowers, production quality and reaction to the environment. However, few efforts had been devoted to improve the quality and longevity of the leaves. Extended greenness in ornamental plants should be a well-established objective in crop breeding.

CHAPTER 3
EFFECT OF CULTIVAR SELECTION, FERTILIZER LEVEL, BUD REMOVAL AND STORAGE CONDITIONS ON POSTPRODUCTION LEAF QUALITY OF POTTED *DENDRANTHEMA GRANDIFLORA* RAMAT TARA AND BOALDI

Introduction

Senescence of potted plants is cultivar and/or clone dependent (Cushman et al., 1994; Molinar and Williams, 1977; Nell and Barrett, 1986b; Nell et al., 1990; Ottosen and Høyer, 1988; Scott et al., 1982). However, the final quality and longevity of potted plants when they reach the consumer is the result of an interaction of variety selection, environmental factors, cultural practices and postproduction handling conditions (Nell, 1991).

Premature leaf yellowing in some potted chrysanthemum cultivars during shipping has been observed by growers (Carl Scharfenberg, Yoder Brothers, Inc., personal communication), and it has become one of the most unexplainable chrysanthemum postproduction problems. The fact that the conditions under which the problem is reported varies for different growers under different cultural and transport situations has made this problem a difficult one to solve. Numerous stresses arising during crop production, storage and transport might induce premature leaf senescence in potted plants.

High fertilizer rates applied during production is one of the factors known to adversely influence postproduction quality in cut and potted chrysanthemums (Kofranek, 1980; Nell et al., 1989; Roude et al., 1991). In cut chrysanthemums, high nitrogen rates produced thick, crinkled, and brittle leaves (Lunt and Kofranek, 1958), and more succulent foliage with increased susceptibility to *Botrytis cinerea* (Waters, 1965; Wesemberg and Beck, 1964). While the requirement for fertilizer is high during the first half of the growing season, sustained high levels of fertilizer until flowering have been shown to reduce flower longevity and leaf quality (Carver et al., 1993; Ishida et al., 1983; Nell et al., 1989). Postproduction injury symptoms in cut chrysanthemum leaves include thickening, downward rolling of margins, chlorotic mottling, bronzing and necrosis of small areas (Waters, 1964; Woltz, 1968, 1969; Woltz and Engelhard, 1971; Woltz and Waters, 1967). In 'Torch' and 'Spirit' potted chrysanthemums grown under high nutrient levels (400 mg $N \cdot l^{-1}$, 20N-4.7P-16.6K), the principal symptom of postproduction decline was a permanent loss of turgor, even when adequate moisture was provided in the media (Carver et al., 1993).

Bud removal and decapitation have been shown to delay leaf senescence in tomato and tobacco (Colbert and Beever, 1981) and in *Phaseolus vulgaris* (Van Staden and Carmi, 1982), and to increase the concentration and total flux of cytokinins in the bleeding xylem sap. Van Staden and Carmi (1982) suggested that

the increase in cytokinin production and supply to the shoot could be due to the higher root to shoot ratio. Traditionally, potted chrysanthemums are 'disbudded': lateral flower buds are removed from each stem to allow only the terminal flower to develop. The result is a plant with one large terminal flower per stem. In order to reduce labor costs, growers are more often using a 'center bud removal' technique where only the terminal bud on each stem is removed, which allows all lateral buds to develop (multiflowered plant). It is possible that the type of bud removal in potted chrysanthemum could affect the development of premature leaf senescence.

Temperature management is the predominant method used to maintain the quality of flowering crops during storage and transport. Reductions in temperature can substantially reduce the rates of many metabolic processes which lead to the natural deterioration and loss of quality (Côme, 1991). Optimum shipping temperatures vary considerably among genera and is also dependent on shipping duration (Poole and Conover, 1983; Sterling and Molenaar, 1986). Sterling and Molenaar (1986) studied 20 economically important potted plants subjected to six storage periods (1-21 days) and six temperatures (5-30°C). They reported that a wide range of temperatures gave optimum storage when the plants were stored for a relatively short period, but a progressively smaller range was best as the products were stored for a longer time. All plants were sensitive to high temperature

and most plants were sensitive to chilling temperatures. For chrysanthemum hybrids the optimum shipping condition was 1 day at 10°C. Nell et al. (1989) found that storage duration had little effect on 'Mountain Peak' chrysanthemum at 4 and 16°C but longevity decreased by 7 days when duration was extended from 2 to 7 days at 24°C.

Preliminary screening experiments conducted with several chrysanthemum cultivars indicated that Tara developed leaf marginal dryness and/or chlorosis and that Boaldi did not develop chlorosis. These two cultivars were chosen to characterize postproduction leaf quality in potted chrysanthemums and to elucidate the possible stresses that may trigger premature leaf senescence. The effect of cultivar, fertilizer level, bud removal and storage duration and temperature were tested in a series of experiments.

Materials and Methods

General Cultural Procedures and Postproduction Conditions

Rooted chrysanthemum cuttings received from a commercial propagator (Yoder Brothers, Inc., Barberton OH) were planted in 11.25-cm (640 cm^3) plastic pots using commercial potting media (Vergro Klay Mix, Verlite Co. Tampa, Fl.). Plants were placed under a noninductive photoperiod (incandescent lamps from 2200 to 0200 hr) in a fiberglass, fan-and-pad cooled greenhouse. The

greenhouse environmental control GEM3™ system (Q-Com, Greenhouse Environmental Manager System, Irvine, Ca) was programmed to vent at 25°C, and heat at 18°C. Four days after planting, the plants were manually pinched and 3 days later the photoinductive period (black cloth pulled over plants at 1700 h and removed at 0800 h) was initiated. Ten days after pinching plants were pruned to three lateral shoots. Butanedioic acid mono (2,2-dimethylhydrazide) (daminozide) was applied at 2,500 $mg \cdot l^{-1}$ as a foliar spray 20 days after planting when lateral shoots were approximately 10 cm long. Each lateral shoot was disbudded (all lateral buds removed) as soon as was physically possible, unless otherwise indicated, to provide one flower per lateral shoot.

When treatments were applied in the greenhouse, experiments were arranged in a randomized complete block design with three replications (unless otherwise specified) with two plants per experimental unit. At flowering, plants were placed in plastic sleeves, boxed and stored in the dark as specified in each experiment. After storage, the plants were placed in interior holding rooms held at 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of PPFD from cool-white fluorescent lamps for 12 hours/day. Temperature was 21±1°C and relative humidity (RH) 50±5%.

After one or two weeks in the interior holding rooms (specified in each experiment), visual leaf quality was rated from 1 to 5 as follows: 1=plants with ≥ 50% chlorotic, necrotic or wilted leaves. These plants were severely damaged. 2=plants

with ≤ 50% and ≥ 25% chlorotic, necrotic or wilted leaves. A visual quality value of 2 represented a plant of unacceptable quality with no longer ornamental value for the consumer. 3=plants with ≤ 25% and ≥ 15% chlorotic, necrotic or wilted leaves. A plant with a value of 3 had visible leaf damage but was still acceptable for the consumer. 4=plants with ≤ 15% chlorotic or necrotic leaves. A value of 4 represented a plant that was not longer a perfect product, but had no evident damage. 5=no chlorotic or necrotic leaf margins.

The data were analyzed using analysis of variance procedure and orthogonal comparisons or the tdiff option in SAS (SAS Institute, Inc., Cary, NC.) was used to examine differences among treatment means.

Experimental Procedures

Experiment 1. Cultivar and complete fertilizer level

This experiment was conducted twice, once in spring 1995 and again in spring 1996. The same treatments were applied during production. At planting, a 3 x 2 factorial combination was established using two cultivars, Tara and Boaldi, and three fertilizer levels (in $mg \cdot l^{-1}$): low (75 N, 18 P and 62 K), medium (150 N, 35 P and 124 K), and high (300 N, 72 P and 248 mg K) from 20N-4.7P-16.6K (60% nitrate, 40% ammoniacal) water soluble fertilizer (Peters, Scotts-Sierra Horticultural Products Co., Marysville, OH). Electrical conductivity of the fertilizer

solutions were 0.79, 1.38 and 2.35 dS·m-1, respectively. Fertilizer treatments were applied at every irrigation throughout production. Planting dates, flowering dates, average daily maximum irradiance, and average daily maximum and minimum temperatures are shown in table 3.1.

At flowering, two plants per treatment per replicate were used to measure media electrical conductivity with the pour-through extraction procedure according to Wright (1986).

Table 3.1. Experimental conditions. Experiment 1.

Planting date	Flowering date	Max PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Max/min Temp (°C)	Storage
6 April 1995	2 June	700	31/20	0,3,6 days @ 21°C
19 April 1996	9 June	671	30/21	2,4,6 days @ 4,12 or 21°C

After storage plants were placed in interior holding rooms, and evaluated two weeks after flowering in 1995. In 1996 plants were evaluated one day after unboxing due to the great injury observed in some treatments. Postproduction evaluation data were analyzed as a 3 x 2 x 3 factorial in 1995 and as a 3 x 2 x 3 x 3 factorial in 1996 to include the three storage temperatures used.

Experiment 2. Storage temperature and duration

Rooted cuttings of Tara and Boaldi were planted on March 15, 1996 and received (in $mg \cdot l^{-1}$) 300 N, 72 P and 249 mg K from 20N-4.7P-16.6K (60% nitrate, 40% ammoniacal) water soluble fertilizer

(Peters, Scotts-Sierra Horticultural Products Co., Marysville, OH). Average maximum irradiance at noon was 655 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and average maximum day and night temperatures were 28 and 17°C, respectively. At flowering on May 10, a 2 x 3 x 3 factorial experiment was established with two cultivars, three dark storage durations, and three temperatures. Plants were stored at 12, 21 or 29°C for 2, 4 or 6 days. Plants were then placed in interior holding rooms and visual quality was rated two weeks after flowering.

Experiment 3. Fertilizer duration and bud removal

A factorial experiment with two fertilizer schedules and two types of bud removal in a randomized complete block design with four replicates was established using Tara. The axillary buds (disbudded plant) or the apical bud (multiflowered plant) were removed as soon as physically possible. Fertilization was either continued throughout production with (in $mg \cdot l^{-1}$) 300 N, 72 P and 249 mg K (20N-4.8P-16K) at every watering (250 ml/pot) or terminated at the time of bud removal. The experiment was repeated twice. Planting dates, flowering dates, average daily maximum irradiance, average daily maximum and minimum temperatures, and total amount of nitrogen applied are shown in table 3.2. Plants were fertilized 13 times and 9 times in the April and August experiment, respectively, before the buds were removed. Plain water was applied afterwards to plants in which fertilizer was terminated at bud removal. During the April experiment fertilizer

solution was applied 30 times to disbudded plants and 36 times to multiflowered plants. In the August experiment fertilizer solution was applied 17 times and 20 times to disbudded and multiflowered plants, respectively. The leachate fraction was approximately one third of the nutrient solution applied per pot (250 ml).

At flowering, two plants per treatment per replicate were used to measure media electrical conductivity with the pour-through extraction procedure according to Wright (1986).

Table 3.2. Experimental conditions. Experiment 3.

Planting date	Bud removal	Flowering date	Max PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Max/min Temp (°C)	Total N (mg)	
					Fert Cont	Fert Term
27 April 1996	Lateral	29 June	820	32/21	2250	975
	Apical	5 July			2700	
26 August 1996	Lateral	23 October	647	30/20	1275	675
	Apical	28 October			1500	

Plants in the April study were stored in the dark at 21°C for 4 days. In the August study, plants were stored for 6 days at 4, 12 or 21°C. Plant longevity (days from flowering) was determined when the outer florets became translucent, and loss of turgor occurred in at least 50% of the flowers, or when 40 to 50% of the foliage turned chlorotic or necrotic. The number of chlorotic or necrotic leaves was recorded at plant death.

Results

Experiment 1. Cultivar and Complete Fertilizer Level

In both years, at the end of production, Tara and Boaldi leaves tended to roll downward with the highest fertilizer level. Leaves in Tara became very thick and succulent, and Boaldi leaves were brittle and broke easily. In addition, some marginal browning or dryness appeared on the leaves in Tara. These dry areas tended to increase during dark storage and over time in the interior holding rooms. Soluble salts in the soil media increased with fertilizer level in Boaldi and Tara (Table 3.3, 1995) reaching values of 5.06 and 5.30 $dS \cdot m^{-1}$, respectively, and values of 4.52 (Table 3.4, 1996). Increase in the soil media soluble salts with increasing fertilizer level has been reported in other studies (Carver et al., 1993; Roude et al., 1991; Yelanich and Biernbaum, 1990). In those studies, values above 3.0 $dS \cdot m^{-1}$ were negatively correlated with plant longevity.

In 1995, the cultivar x fertilizer x storage duration interaction was not significant. Postproduction leaf quality of both cultivars, evaluated two weeks after flowering, decreased as fertilizer rate increased (Table 3.3). However, the symptoms were different. Chlorosis developed in Tara leaves for plants given the highest fertilizer level (Figure 3.1, A), but Boaldi leaves became dry (bottom leaves) and never showed chlorosis (Figure 3.1B). Tara

was more affected by storage duration than Boaldi (Table 3.3). No chlorosis was observed in Tara stored for 0 or 3 days. After 6 days of dark storage, Tara leaves developed chlorosis rapidly and the leaves wilted within a week in the interior holding rooms (Figure 3.1C, right). After only one week in the interior holding rooms, plants grown with the highest fertilizer level and stored for 6 days at 21°C had no ornamental value for the consumer. In Boaldi, leaf quality was not affected by storage duration (Table 3.3).

In 1996, postproduction leaf quality was not affected by the cultivar x fertilizer x storage temperature x storage duration interaction. However, there was a significant temperature x fertilizer interaction (Table 3.4), as well as a cultivar x temperature x storage duration interaction (Table 3.5). After storage at 21 and 29°C, low quality ratings were associated with high fertilizer level, and symptoms included marginal leaf dryness and chlorosis in Tara (Figure 3.1 D). These symptoms were similar to those observed in Tara in the 1995 experiment. These plants, evaluated one day after unboxing, had visible leaf injury symptoms but had still ornamental value for the consumer. In Boaldi, the low leaf quality ratings associated with high fertilizer level were due to bottom dryness of leaves, but they always maintained a dark green color in the interior holding rooms.

Tara showed a marked sensitivity to 12°C storage in this experiment. The low quality ratings were due to wilted and

Table 3.3. Interaction of fertilizer level (F) and cultivar (C) on soil media electrical conductivity (EC) at flowering. Effect of fertilizer, cultivar and storage duration (D) on postproduction leaf quality. Plants were stored at 21°C (Experiment 1, 1995).

	EC ($dS \cdot m^{-1}$)		Leaf Quality Rating[z]
	Cultivar (C)		
Fertilizer(F)	Boaldi	Tara	
Low	0.89	0.93	4.2
Medium	3.15	2.40	3.7
High	5.06	5.30	2.9
Linear[y]	0.001	0.001	0.001
Quadratic[x]	0.001	0.918	NS
F x C[w]	0.0012		

Storage(D) (days)	Cultivar (C)	
	Boaldi	Tara
0	4.1	4.2
3	3.7	3.7
6	3.6	2.2
Linear[y]	NS	0.001
Quadratic[x]	NS	0.039
C X D[v]	0.001	

[z],Quality rating was evaluated 2 weeks after flowering,
1= severely damaged.
2= unacceptable quality, no ornamental value for the consumer.
3= visible leaf damage, but still acceptable for the consumer.
4= no longer a perfect product, but had no evident damage.
5= no damaged leaves.
[y],[x] P value for the linear and quadratic orthogonal contrast, respectively. Nonsignificant (NS) at $P \geq 0.05$.
[w], P value for the fertilizer and cultivar interaction
[v], P value for the cultivar and storage duration interaction.

Table 3.4. Effect of fertilizer and cultivar on soil media electrical conductivity (EC). Interaction effect of fertilizer (F) and storage temperature (T) on postproduction leaf quality. (Experiment 1, 1996).

	EC ($dS \cdot m^{-1}$)	Leaf Quality Rating[z]		
		Temperature (T) (°C)		
Fertilizer (F)		12	21	29
Low	1.02	3.2	4.2	4.2
Medium	2.84	3.1	3.9	3.9
High	4.52	2.5	3.1	2.9
Linear[y]	0.001	0.001	0.001	NS
Quadratic[x]	0.584	0.006	0.012	0.045
Pr>F	0.001			
Cultivar				
Boaldi	3.21			
Tara	3.01			
Pr>F	0.0201			
F x T[w]			0.018	

[z],Quality rating was evaluated one week after flowering,
1= severely damaged.
2= unacceptable quality, no ornamental value for the consumer.
3= visible leaf damage, but still acceptable for the consumer.
4= no longer a perfect product, but had no evident damage.
5= no damaged leaves.
[y],[x],P value for the linear and quadratic orthogonal contrast, respectively. Nonsignificant (NS) at $P \geq 0.05$.
[w], P value for the fertilizer and temperature interaction.

Table 3.5. Interaction of cultivar (C), temperature (T), and storage duration (D) on postproduction leaf quality. (Experiment 1, 1996).

Temperature(T) (°C)	Storage Duration (D) (Days)	Leaf Quality Rating[z] Cultivar (C)	
		Boaldi	Tara
12	2	3.7	3.6
	4	3.9	1.5
	6	3.8	1.0
Linear[y]		NS	0.001
Quadratic[x]		NS	0.001
21	2	3.8	3.8
	4	3.6	3.8
	6	3.5	3.7
Linear[y]		NS	NS
Quadratic[x]		NS	NS
29	2	3.6	3.8
	4	3.8	3.7
	6	3.5	3.4
Linear[y]		NS	NS
Quadratic[x]		NS	NS
T x D x C[w]		0.001	

[z],Quality rating was evaluated one week after flowering,
1= severely damaged.
2= unacceptable quality, no ornamental value for the consumer.
3= visible leaf damage, but still acceptable for the consumer.
4= no longer a perfect product, but had no evident damage.
5= no damaged leaves.
[y],[x], P value for the linear and quadratic orthogonal contrast, respectively. Nonsignificant (NS) at $P \geq 0.05$.
[w], P value for the temperature, storage duration and cultivar interaction.

Figure 3.1. Experiment 1. 1995: A) Marginal leaf necrosis and initial chlorosis in 'Tara' associated with high fertilizer rates after 6 days storage at 21°C. B) 'Boaldi' two weeks after flowering (6 days storage at 21°C). C) 'Tara' plants stored for 0, 3 and 6 days at 21°C, two weeks after flowering.
1996: D) Initial chlorosis in 'Tara' leaves after storage for 6 days at 29°C. E)'Tara' leaves after storage at 12°C for 6 days.


A


B


C


D


E

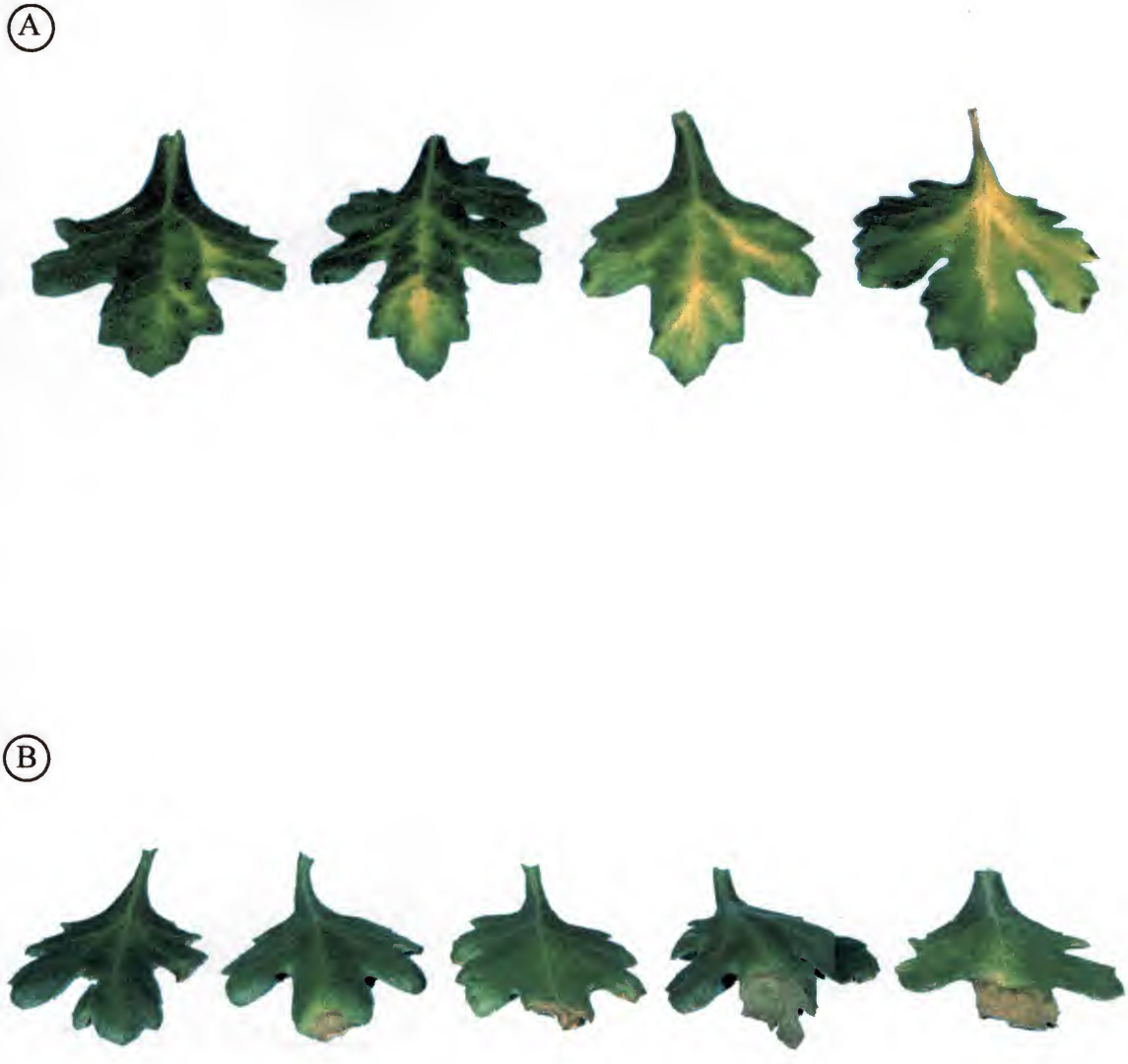

Figure 3.2. 'Tara' A) Pattern of leaf veinal chlorosis in plants stored at 12°C. B) Pattern of marginal necrosis associated with high fertilizer rate.

chlorotic leaves when the plants were unboxed (Figure 3.1, E). These symptoms were also evident in plants grown at the lowest fertilizer level, although increasing fertilizer resulted in greater injury and plants of unacceptable quality (Table 3.4). Leaf quality in Boaldi was not affected by storage temperature or duration (Table 3.5). Increasing storage duration at 21 and 29°C did not affect Tara leaf quality (Table 3.5). These results were different from those found in 1995 (Table 3.3). In this experiment, however, increasing storage duration at 12°C greatly reduced leaf quality in Tara (Table 3.5). Plants stored for 4 and 6 days at 12°C had no ornamental value one day after unboxing.

The most characteristic symptoms of leaf injury in Tara after storage at 12°C were the loss of turgor in the upper leaves, followed by the appearance of chlorosis. Lower leaves tended to develop a veinal chlorosis (Figure 3.2 A) and eventually all leaves wilted. Leaf injury symptoms in Tara stored at 21 or 29°C are generally associated with higher fertilizer rates, and are characterized by dry necrotic margins (Figure 3.2 B) from which chlorosis developed (Figure 3.1 A,D).

Experiment 2 - Storage Temperature and Duration

While Tara was more susceptible to prolonged dark storage than Boaldi (Table 3.6), the results of this experiment were different from those in experiment 1. No major chlorosis was observed in this experiment. Injury symptoms in Tara included

slight chlorotic points around the margins of leaves that were usually associated with dry and brown tips. Storage temperature did not affect the leaf quality in this experiment. The results of this experiment are typical of the other winter experiments (see Appendix B), in which leaf quality was not significantly affected by storage temperature or duration.

Table 3.6. Interaction of storage duration (D) and cultivar (C) on postproduction leaf quality. (Experiment 2).

Cultivar	Storage Duration(D) (days)	Leaf Quality Rating[z]
Boaldi	2	3.8
	4	3.8
	6	3.9
Linear[y]		NS
Quadratic[x]		NS
Tara	2	3.9
	4	3.9
	6	3.3
Linear[y]		0.016
Quadratic[x]		NS
C x D[w]		0.025

[z],Quality rating was evaluated two weeks after flowering,
1= severely damaged.
2= unacceptable quality, no ornamental value.
3= visible leaf damage, but still acceptable for the consumer.
4= no longer a perfect product, but had no evident damage.
5= no damaged leaves.
[y],[x],P value for the linear and quadratic orthogonal contrast, respectively. Nonsignificant (NS) at $P \geq 0.05$.
[w],P value for the cultivar and storage duration interaction.

Experiment 3. Fertilizer Duration and Bud Removal

Postproduction longevity of Tara was one week longer when plants were disbudded compared to multiflowered plants, and continuing fertilization until flowering decreased longevity by 4.7 days compared to fertilizer termination (Table 3.7). Reduced longevity in multiflowered plants was due to decreased flower longevity and to a marked development of veinal leaf chlorosis (Table 3.7). Chlorosis was not apparent at unboxing, but developed within 1 or 2 days in the interior holding rooms. Veinal chlorosis developed first, followed by the appearance of dry/necrotic areas (Figure 3.3); leaves eventually wilted, turned brown and became dry.

Continued fertilizer application until flowering affected both plant types but caused different symptoms. In multiflowered plants, continued fertilization accelerated the development of the symptoms described above (Figure 3.4 A, right), and on disbudded plants, marginal dryness of the bottom leaves resulted (Figure 3.4 B, right). When fertilizer was terminated, all plants had fewer chlorotic and necrotic leaves (Table 3.7), and disbudded plants showed no leaf injury (Figure 3.4 C, left). As expected, soluble salts of the media were markedly lower when fertilizer was terminated at bud removal (Table 3.7). When fertilizer was applied until flowering, soluble salts levels were higher in the soil of multiflowered plants than in disbudded plants (Table 3.7).

Table 3.7. Effect of fertilizer (F) continuation (Cont) or termination (Term) and type of bud removal(B) on soil media electrical conductivity (EC) of Tara at flowering, and on plant longevity, number of upper and lower chlorotic leaves and number of lower necrotic leaves at plant death. (Experiment 3, April, 1996).

	EC[z] ($dS \cdot m^{-1}$)		Longevity[y] (days)	Number of low chlorotic leaves	Number of upper chlorotic leaves	Number of low necrotic leaves
	Fertilizer					
Type	Cont	Term				
Disbudded	2.58b	0.43c	21.5	0.9	0.2	5.1
Multiflowered	3.26a	0.48c	13.3	2.1	2.5	6.6
Pr > F			0.001	0.026	0.001	NS
Fertilizer						
Cont			15.1	2.8	2.3	8.7
Term			19.8	0.3	0.4	3.1
Pr > F			0.001	0.001	0.028	0.001
F x B[x]	0.004		NS	NS	NS	NS

[z],Lsmean separation, tdiff option in SAS: values followed by the same letter are not significantly different at P≥0.01.
[y], Number of days from flowering.
[x] ,P value for the fertilizer and type of bud removal interaction. Nonsignificant (NS) at P≥0.05.

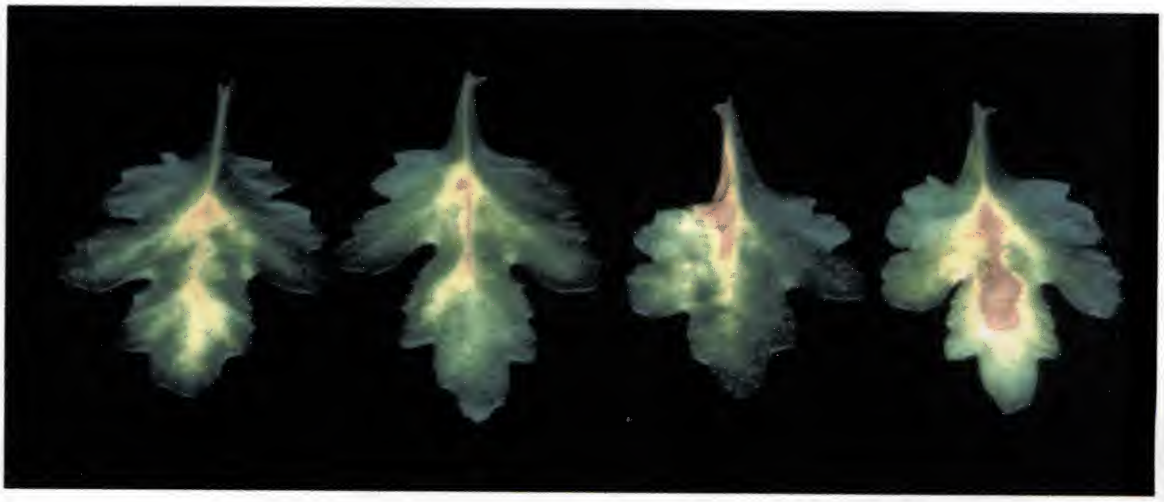

Figure 3.3. Experiment 3. Pattern of development of leaf veinal chlorosis and necrosis in multiflowered 'Tara'.

Figure 3.4. Leaf chlorosis and bottom leaf necrosis in 'Tara'. A) multiflowered plants fertilized until flowering (right), fertilizer termination (left). B) Leaf chlorosis in multiflowered (left) and bottom leaf necrosis in disbudded plants (right). C) Disbbuded plants, fertilizer termination (left), fertilizer continuation (right).


A


B


C

Multiflowered plants flowered a week later than disbudded plants. Because plants received fertilizer at every watering, more fertilizer was applied to multiflowered plants which could explain the higher salts. Nevertheless, the fact that multiflowered plants fertilized only until bud removal (very low media EC) also developed leaf chlorosis strongly indicates that a factor other than high soluble salts and/or high fertilizer rate caused the leaf chlorosis in this experiment. The fact that multiflowered plants developed a distinctive pattern of chlorosis after only 4 days of storage suggests that manipulation of the shoot had a important effect on the postharvest leaf longevity.

Repetition of the experiment in August 1996 included three storage temperatures: 4, 12 and 21°C to test the susceptibility of the cultivar Tara to 12°C observed in experiment 1 (1996). Postproduction longevity of plants stored at 12°C was reduced by 5.4 days compared to plants stored at 4°C, regardless of the type of bud removal and fertilizer treatment (Table 3.8). The first symptoms of injury at unboxing was an initial loss of turgor in some leaves on plants stored at 12°C (Figure 3.5 A). Loss of turgor appeared first in the distal part of upper leaves and progress in some lower leaves at the same time that chlorosis developed (Figure 3.5B). These results were similar to those found in experiment 1 (1996).

The fertilizer and temperature interaction showed that more leaves were damaged at 21°C when fertilizer was applied until

Table 3.8. Effect of storage temperature (T), type of bud removal and fertilizer (F) continuation (Cont) or termination (Term) on Tara plant longevity and total number of damaged leaves. Plants were stored in the dark for 6 days (Experiment 3, August 1996).

	Longevity[z]	Number of Leaves Damaged	
Type			
Disbudded	20.6	6.5	
Multiflowered	18.8	13.3	
Pr>F	0.051	0.005	
		Fertilizer (F)	
Temperature[z] (T)		Cont	Term
4	22.3	5.5	3.1
12	16.9	13.9	12.8
21	20	13.8	1.2
Linear[y]	0.048	0.002	NS
Quadratic[x]	0.001	NS	0.001
F X T[w]		0.001	

[z],Number of days from flowering.
[y],[x],P value for the linear and quadratic orthogonal contrast, respectively. Nonsignificant (NS) at P≥0.05.
[w],P value for the fertilizer and temperature interaction.

flowering compared to fertilizer termination (Table 3.8). Overall, multiflowered plants had more damaged leaves than disbudded plants (Table 3.8). Similar results were observed in the April experiment. The pattern of veinal chlorosis in fully turgid leaves was similar to the pattern observed in previous experiments. Plants stored at 4°C had no chlorotic leaves, and dry leaf margins

Figure 3.5. A) Initial loss of turgor in 'Tara' leaves stored for 6 days at 12°C, right after unboxing; B) one day after unboxing; C) wilting and chlorosis in disbudded plants 7 days after unboxing and D) wilting and chlorosis in multiflowered plants one day after unboxing.

appeared only in some plants. Plants stored at 4°C lasted longer than plants stored at 12 or 21°C and showed no major leaf injury.

Discussion

Of the factors evaluated, cultivar selection had the greatest influence on chrysanthemum postproduction leaf quality. This fact has been emphasized in several previous studies (Cushman et al. 1994; Nell et al. 1990; Ottosen and Høyer, 1988). Boaldi leaves are dark green, and never showed chlorosis regardless of the treatments imposed. However, Tara appeared to be susceptible to a variety of production and storage conditions, and often the interaction of both triggered premature leaf chlorosis. Thus, there appears to be two different problems: One is the production and shipping conditions that negatively affect postharvest leaf quality in Tara, and the other is a genetic difference between the two cultivars in their rate or pattern of senescence.

High fertilizer levels during production clearly reduced leaf quality (Experiment 1). These results are similar to those reported for other chrysanthemum cultivars (Carver et al., 1993; Ishida et al., 1983; Lunt and Kofranek, 1958; and Nell et al., 1989). Penning de Vries (1975) emphasized that salinity induced by heavy fertilization generally stimulates processes for maintenance of ion concentration. At relatively high night temperatures during crop production, the maintenance cost is large and the pool of

reserve carbohydrates can be depleted before the end of the night (Scharz and Gale, 1983). Leaf chlorosis was induced in Tara in experiments conducted in late spring and early fall when maximum average temperature during production reached 30°C and minimum average temperature was 20°C. An increased volume of fertilizer solution applied (especially with the highest level) due to a higher evaporative demand, and elevated temperatures during production could result in the depletion of carbohydrates in the leaves. Furthermore, prolonged dark storage has been shown to induce carbon starvation and leaf senescence (Krapf and Kacobi, 1975). It is possible that premature chlorosis in Tara (Experiment 1, and Experiment 3) after dark storage at 21 and 29°C is associated with low reserves carbohydrates. Leaf chlorosis in Easter lilies (Jiao et al., 1986; Miller et al., 1995), and leaf blackening in *Protea* (Bieliski et al. 1992; Dai and Paull, 1995; McConchie and Lang, 1993) have been related to reduced stored carbohydrates.

Tara showed a susceptibility to storage at 12°C (Experiment 1, 1996 and Experiment 3, August), regardless of the fertilizer rate and type of bud removal. Loss of leaf turgor and wilting were observed when the plants were unboxed. The fact that loss of turgor and wilting occurred after only two days of storage at 12°C (plenty of moisture in the soil), and that the symptoms also developed in plants with the lowest fertilizer level, indicates that leaf wilting was not caused by a high soluble salts in the

soil media (low osmotic potential). Moreover, leaves with only a slight loss of turgor when the plants were unboxed did not recover turgor after watering. Even though these symptoms seem to be caused by a localized injury in the leaves, high fertilizer levels could contribute to the greater injury observed after storage at 12°C by decreasing the osmotic potential in the roots and reducing water uptake.

These symptoms are similar to those of chilling injury reported in some studies (Salveit and Morris, 1990; Paull, 1990). However, sensitivity to 12°C was unexpected since there are no previous reports of chilling injury on chrysanthemums. In fact, most frequently, postharvest longevity in chrysanthemums has been reported to decline at higher temperatures, and it has often been recommended to store and transport chrysanthemums at/or below 10°C (Nell et al., 1989; Sterling and Molenaar, 1986; Tijskens et al., 1996). However, the fact that loss of turgor and wilting did not develop when the plants were stored at 4°C (Experiment 3, August) indicates that these symptoms are probably not the result of chilling injury. Loss of turgor and wilting after storage at 12°C was only observed in experiments conducted in late spring and early fall which suggest a possible interaction of production temperature and storage temperature in chrysanthemum that merits further investigation.

Manipulation of the number of flower buds in the plant also affected postproduction leaf chlorosis in Tara (Experiment 3).

Multiflowered plants developed a marked veinal chlorosis after 4 days of dark storage at 21°C, while disbudded plants did not. These results are similar to those reported by Colbert and Beever (1981) and Van Staden and Carmi (1982). Leaf senescence can be delayed or even reversed if buds and other plant parts are removed (Noodén, 1990). This process has been studied extensively in *Perilla frutescens* (Beever and Woolhouse,1974), tomato and tobacco (Colbert and Beever, 1981) and *Phaseolus vulgaris* (Van Staden and Carmi, 1982). These authors reported an increase in the root to shoot ratio in disbudded plants compared to intact plants, and an enhanced cytokinin supply from the roots to the rest of the plant. A decrease in cytokinin production in the roots could be involved in inducing premature leaf senescence in multiflowered Tara plants.

In this study, several treatments during production and storage induced at least three different leaf injury symptoms in Tara, which eventually resulted in premature leaf senescence. 1) Dryness and necrosis of bottom leaves, and marginal necrosis and chlorosis of upper leaves were associated with high fertilizer levels (Figures 3.1 A, D; Figure 3.2 B; Figures 3.4 B, C, right). Under prolonged dark storage conditions chlorosis eventually can affect all leaves (Figure 3.1, C, right). 2) Loss of turgor and wilting were the first observed symptoms of injury after storage at 12°C (Figures 3.5 A, B, C). 3) Veinal leaf chlorosis was the characteristic pattern in leaves of multiflowered plants after

dark storage at 21°C (Figure 3.2 A; Figure 3.3; Figures 3.4 A, B, left), and was also observed in both types after storage at 12°C (Figure 3.1, E; Figure 3.5, D). All these symptoms were observed in experiments conducted either in late spring or early fall.

This study showed that season, fertilizer regimen, type of bud removal, and storage temperature and duration might affect the development of premature leaf senescence in a susceptible cultivar as Tara, which explains why different growers claimed problems with leaf yellowing under different cultural and transport conditions.

The most striking difference in the postharvest leaf performance of both cultivars is the fact that chlorosis was never observed in Boaldi leaves regardless of the treatments imposed. Boaldi leaves eventually dry on the plant. This observation suggests that Boaldi may be a type of stay-green phenotype, which has been described in several crops (Thomas and Smart, 1993). The comparative study of the senescence pattern in detached dark-induced leaves of both cultivar is the subject of the next study (Chapter 4).

CHAPTER 4
DARK-INDUCED LEAF SENESCENCE IN *DENDRANTHEMA GRANDIFLORA* RAMAT TARA AND BOALDI

Introduction

In many species, the most obvious symptom of senescence is yellowing of leaves resulting from degradation of chlorophyll (Chl), localized exclusively in the thylakoid membranes (Thimann, 1980). The net loss of protein has also been recognized as a dominant factor of senescence (Peoples and Dalling, 1988). Under most circumstances, degradation of chlorophyll and protein during senescence proceeds in a coordinated manner, implying a corresponding coordination in the breakdown of stroma and lamellae (Thomas and Stoddart, 1980). In consequence, Chl concentration has been employed widely as an index of leaf senescence, not only in the study of senescence *per se*, but also in the bioassay of giberellins (White, 1966), cytokinins (Osborne and McCalla, 1961), and other growth regulators which influence senescence. In such studies, it was commonly assumed that Chl degradation is linked in an obligate fashion to all the other components of the senescence process.

In 1975, Thomas and Stoddart described a non-yellowing mutant of *Festuca pratensis* L. in which the linkage between

chlorophyll degradation and proteolysis had been broken. Genotypes with leaves which remain green longer than normal, termed *stay-green* varieties, have been found in a wide range of species (Thomas and Smart, 1993). Some of these mutants retained both chlorophyll and photosynthetic competence ('functional' *stay-green*). Others keep chlorophyll but degrade some other components of the photosynthetic apparatus (non-functional *stay-green*). Functional stay-green genotypes show either delay initiation of senescence (Phillips et al., 1984) or a reduction in its rate of progress (Crafts-Brandner et al., 1984). Very detailed studies of the chloroplast of non-yellowing *Festuca* (non-functional stay-green) leaf tissue showed that stroma components, grana stacks and photoreduction capacity declined during senescence, whereas thylakoid membranes persisted and retained a number of structural proteins (Thomas, 1977, 1982a). In this case the capacity of the mutant to dismantle its thylakoids is in some way impaired.

This study describes some of the physiological and biochemical changes occurring during dark-induced senescence in detached and attached leaves of two *Dendranthema grandiflora* cultivars, Boaldi, a non-yellowing genotype and Tara, a normal yellowing genotype. The effect of exogenous ethylene, sucrose and cytokinins on leaf senescence of these two cultivars was also investigated.

Materials and Methods

Plant Material and General Cultural Procedures

Boaldi and Tara chrysanthemum cuttings received from a commercial propagator (Express Seed Co., Berlin, OH) were planted in 11.25 cm (640-cm^3) plastic pots using commercial potting media (Vergro Klay Mix, Verlite Co., Tampa, Fla.). At every watering, plants received (in $mg \cdot l^{-1}$) 300 N, 72 P and 249 K from 20N-4.7P-16.6K (60% nitrate, 40% ammoniacal) water soluble fertilizer (Peters, Scots-Sierra Horticultural Products Co.). Plants were placed under a noninductive photoperiod (incandescent lamps were used from 2200 to 0200 hr), unless otherwise indicated, and manually pinched four days later. Seven days after planting the photoinductive period (black cloth pulled over plants at 1700 hr and removed at 0800 hr) was initiated. Ten days after pinching, plants were pruned to three lateral shoots. Butanedioic acid mono (2,2-dimethylhydrazide) (daminozide) was applied at 2,500 $mg \cdot l^{-1}$ as a foliar spray 20 days after planting, when lateral shoots were approximately 10 cm long. The apical bud was removed in each stem, as soon as was physically possible, to allow all lateral inflorescences to develop. Plants were grown to the flowering stage when treatments were applied.

Detached leaf senescence

Cuttings of both cultivars were planted on June 29 and grown in a temperature-controlled glasshouse held at 23/18°C day/night. At flowering, healthy, fully expanded leaves that constituted the visible canopy were excised from the plant near the base of the petiole. The leaves were placed individually in 250-cm^3 jars with the petioles submerged in a small vial containing approximately 10 cm^3 of deionized water (dH_20). Jars were kept in a dark room at 23±1°C and 50±5% RH with free air circulation for 12 days. Chlorophyll (Chl), protein concentration, and electrolyte leakage were determined at day 0, 3, 6, 9 and 12. Proteolytic activity was determined at day 0, 6, 9, and 12. The experiment was repeated three times (three harvest dates, three days apart). In each experiment, Chl, and electrolyte leakage were measured in three individual leaves at each sample date, and data represent the mean and standard deviation of the means of the three experiments. Total soluble protein and proteolytic activity data represent the mean and standard deviation of three replicates, one per experiment.

Detached versus attached leaf senescence

Rooted cuttings of both cultivars were planted on 27 August in a fan-and-pad-cooled glasshouse. Maximum day and minimum night temperatures were 30/22°C . At flowering, leaves were harvested from a group of plants as described above, while another group

was maintained intact. The leaves in this experiment, probably due to higher temperatures, were larger than in the previous one, and tended to wilt rapidly when placed with the petioles in vials. Therefore they were floated in trays containing dH_20. Detached leaves and whole plants were kept in the dark at 23±1°C and 50±5% RH. Leaves were sampled at day 0, 3, 6, 9 and 12 for chlorophyll and protein concentration, and $C0_2$ evolution.

Exogenous ethylene, sucrose and cytokinins

Rooted cuttings were planted on 24 September in a fan- and-pad-cooled polyethylene covered greenhouse programmed to vent at 25 ±1°C and grown under natural days. At flowering, leaves were harvested and placed in jars with the petioles submerged in a small vial containing approximately 10 cm^3 of dH_20. Leaves were enclosed in 40-L Plexiglas boxes and treated with 100 $\mu l \cdot l^{-1}$ ethylene or air for 3 days in the dark at 23°C. The boxes were opened daily, vented, and reinjected with 4 ml of ethylene. At the end of the treatment, leaves were immediately sampled for chlorophyll concentration.

Another group of leaves were floated in 1% sucrose solution or dH_20. To reduce bacteria growth, the pH of the sucrose solution was decreased to 3.5 with citric acid. Leaves were also floated in another solution of dH_20 at pH 3.5 as control. Chlorophyll concentration was determined initially and at days 3, 6 and 9 in the dark.

Benzyladenine (BA) at 0, 5, 25 and 50 $mg \cdot l^{-1}$ was sprayed on intact plants 1 day before they were placed in the dark. Leaf samples for chlorophyll analysis were taken from attached leaves initially and at days 3, 6, 9, 12.

Experimental Procedures

Electrolyte leakage and chlorophyll concentration

Seven leaf disks (0.785 cm^3-each) per sample were rinsed with dH_20 and placed in vials containing 7 ml dH_20. After an initial measurement of the electrical conductivity (C_{ini}) of the solution, the samples were placed under vacuum at 20 psi to ensure good immersion of the leaf disks, and then placed in a rotatory shaker for 4 h. The electrical conductivity (C_{fin}) was measured at the end of the shaking period and again after samples were frozen and thawed to obtain total conductivity (C_{tot}). Electrolyte leakage (%) was calculated using the formula:

$$EL(\%) = (C_{fin} - C_{ini}) / C_{tot} \times 100$$

Chlorophyll was extracted from ten leaf disks (0.307-cm^2 each) per leaf in ice-cold 80% acetone and determined following procedures established by Bruinsma (1963).

Protein determination and gel electrophoresis

Total soluble protein was extracted from 1 g of leaf tissue in a buffer containing 5% glycerol, 5% β-mercaptoethanol, 1% Sodium dodecyl sulphate (SDS), and 6.25 mM Tris-HCL (pH 6.8). Protein samples were boiled for 2.5 min and clarified by

centrifugation at 5,000 rpm for 25 min. Supernatants were collected and 500 μl of the supernatant were precipitated with 100 μl of ice cold acetone containing 1% β-mercaptoethanol. Samples were centrifuged at 5,000 rpm for 20 min and the precipitated protein resuspended in 100 μl SDS buffer. Proteins were quantified by the method of Bradford (1976) with Bovine Serum Albumin as a standard.

One dimensional gel electrophoresis was carried out according to the method of Laemmli (Ausubel et al., 1989). Forty or fifty μg of protein per lane were loaded (depending on the size of the gel) and resolved on a 5% stacking-10% resolving SDS polyacrylamide gel electrophoresis (SDS-PAGE). Bio-Rad molecular weight standards were used for all gels. Following electrophoresis the gels were stained with 0.025% Coomassie Brilliant Blue for 12h and washed with several changes in a 45% methanol and 10% glacial acetic acid solution.

Proteinase assay

To determine proteolytic activity, 1.5 g of leaf tissue were homogenized in 4 ml buffer containing 150 mM NaCl, 50 mM sodium phosphate (pH 7.2), and 14 mM 2-mercaptoethanol (Beers and Freeman, 1997). After centrifugation at 10,000 rpm for 20 min at 4°C, 500μl of supernatant were added to 100μl of azoalbumin solution (Gerhardt et al., 1994). This substrate consists of a dye that is chemically conjugated to the protein; hydrolysis of the protein results in liberation of the dye. Absorbance of the

supernatant was read at A_{400}. Proteolytic activity was determined at pH 7.5 and 5.5, and is expressed in optical density units per μg of protein.

CO_2 evolution

Leaves were weighed and placed in 250 ml Mason jars with their petioles immersed in a small vial containing 10 ml dH_20. All jars were fitted with a sampling port. Jars were sealed for 4 h to allow CO_2 to accumulate. A 1.0 cm^3 sample of gas was withdrawn just after sealing the jars and 4 h later and analyzed for CO_2 using a Gow Mac gas chromatograph.

Results

Detached Leaf Senescence

Leaves of the yellowing cultivar Tara are distinguished from Boaldi leaves, a non-yellowing cultivar, by the rate at which Chl is lost from excised leaves in the dark. Over a period of 12 days after excision, there was a steady decline virtually to zero in the leaf Chl content of the yellowing cultivar (Figure 4.1), which by day 12 were completely chlorotic (Figure 4.2). On the other hand, in the non-yellowing cultivar, Chl remained at a high level for the 12-day period (Figure 4.1) and visual yellowing was never evident (Figure 4.2).

Electrolyte leakage (EL), expressed as percent of the total leakage, showed a significant increase in the yellowing cultivar

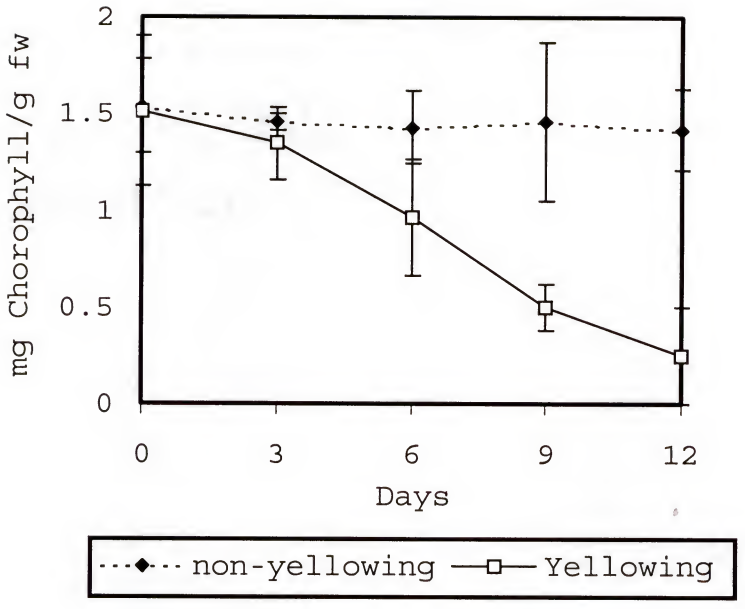

Figure 4.1. Rates of Chlorophyll degradation in detached leaves of a non-yellowing cultivar, Boaldi, and a yellowing cultivar (Tara) during dark-induced senescence. Each point represents the mean ± SD of the means of three experiments.

from day 3 in the dark, but remained at low values in the non-yellowing cultivar during the 12-day period (Figure 4.3). The leakage of electrolytes from leaf tissues, though not frequently used as an indicator of leaf senescence, is a widely accepted indicator of membrane integrity.

Total soluble leaf protein declined in the yellowing cultivar approximately 45% in 12 days (Figure 4.4). In contrast, the non-yellowing cultivar maintained the initial soluble protein concentration over the same period in the dark (figure 4.4). SDS-polyacrylamide gel electrophoresis of soluble protein did not show major differences in banding patterns between the two cultivars at day 0.

Figure 4.2. Leaves of a non-yellowing cultivar Boaldi (top), and a yellowing cultivar Tara (bottom) after 0, 3, 6, 9 and 12 days in the dark.

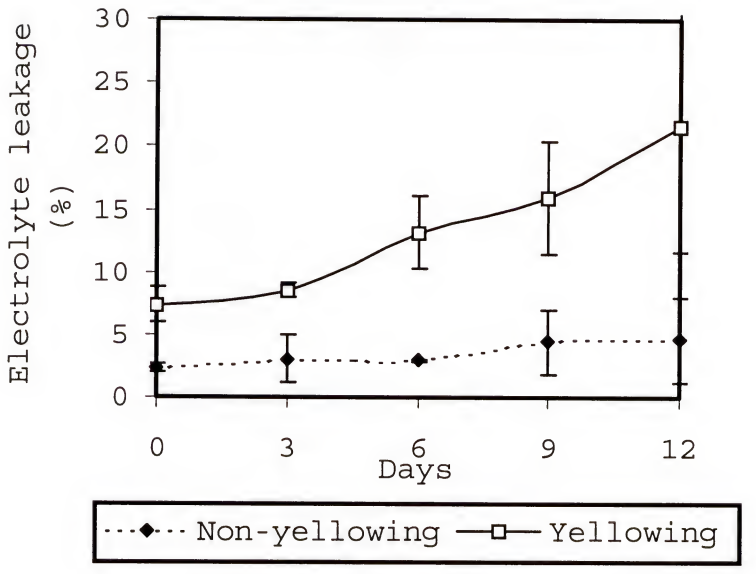

Figure 4.3. Electrolyte leakage, expressed as percent of total leakage in detached leaves of a non-yellowing cultivar Boaldi, and a yellowing cultivar Tara during dark-induced senescence. Each point represents the mean ± SD of the means of three experiments.

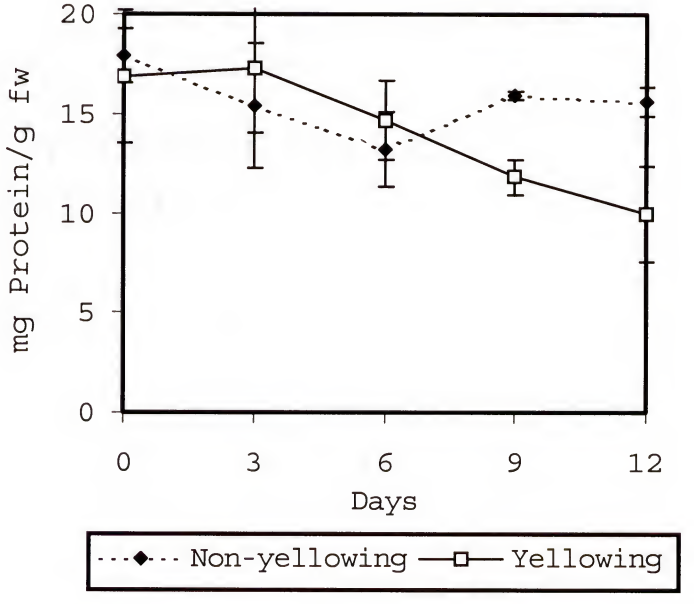

Figure 4.4. Total soluble protein in detached leaves of a non-yellowing cultivar Boaldi, and a yellowing cultivar Tara during dark-induced senescence. Each point represents the mean ± SD of three replicates, one per experiment.

However, all the resolved protein components were lost in the yellowing cultivar by day 12 (Figure 4.5). In the non-yellowing cultivar, on the other hand, the intensity of the bands observed at day 0 was unchanged over the 12 day period (Figure 4.5). There were also major differences between the two cultivars in the proteolytic activity of the leaves during senescence. The level of total acid (pH 5.5) and basic (pH 7.5) azoalbumin hydrolase activities increased with time during senescence in the yellowing cultivar, but remained at the initial level in the non-yellowing cultivar (Table 4.1). Azoalbumin is an artificial substrate for plant proteinases but it is generally believed to be hydrolyzed by the same enzyme or enzymes that act in vivo on soluble proteins such as RuBPC (Thomas and Huffaker, 1981).

Detached versus Attached Leaf Senescence

Chlorophyll concentration declined progressively in the yellowing cultivar in both detached and attached leaves, reaching values as low as 0.3 $mg.g^{-1}$ fresh weight (Figure 4.6). A marked decrease occurred earlier in detached leaves in which chlorosis was evident by day 9. In attached leaves there was a steady decrease and chlorosis was very general on the whole plant by day 12 (Figure 4.7, A).In the non-yellowing cultivar, Chl levels were maintained at the initial high level in detached and attached leaves (Figure 4.6), not only for the 12-day period shown in the diagram, but for as long as the leaves were kept in these

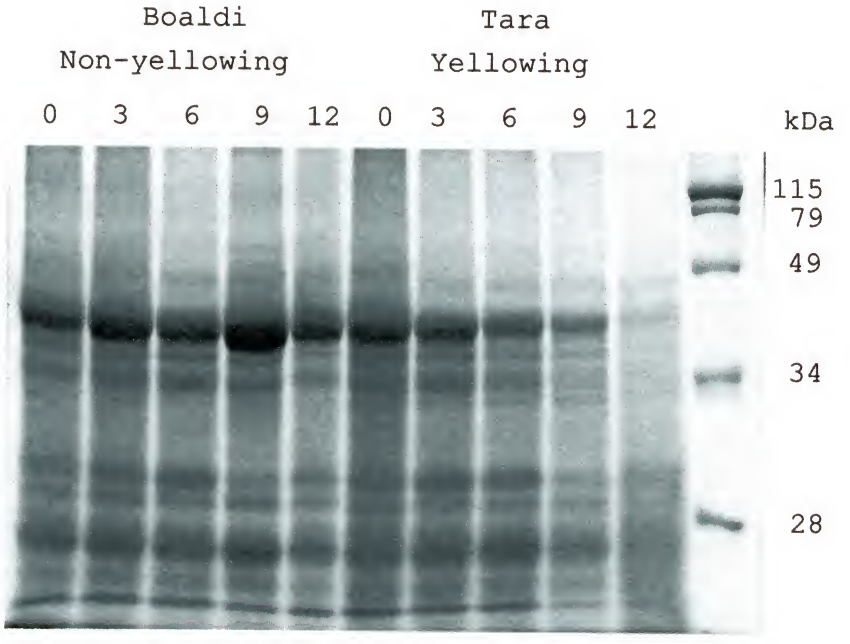

Figure 4.5. SDS-Polyacrylamide gel electrophoresis of total soluble protein in detached leaves of a non-yellowing cultivar Boaldi, and a yellowing cultivar Tara during dark-induced senescence. Numbers on the top of each line represent days in the dark. Molecular weight markers (kDa) are specified in the right line. Each line was loaded with 50 μg protein.

Table 4-1. Proteolitic activity ($A_{400}/\mu g$ protein) in detached leaves of a non-yellowing cultivar Boaldi and a yellowing cultivar Tara during dark-induced senescence. Data represent the mean ± SD of three replicates, one per experiment.

	Non-yellowing		Yellowing	
	pH 5.5	pH 7.5	pH 5.5	pH 7.5
	$A_{400}/\mu g$ protein $x(10^{-5})$			
Initial	4.7±0.6	4.6±0.9	3.7±0.4	3.6±0.4
d=6	7.0±1.9	7.5±1.1	6.1±1.1	6.6±2.0
d=9	6.0±0.3	6.1±0.3	12.2±3.4	11.9±3.1
d=12	6.9±0.4	6.7±0.2	19.9±6.4	19.4±7.2

conditions (18 days). In this experiment, leaves were floated in dH_20, and the non-yellowing cultivar was maintained in the dark for 18 days. At this time, a slight chlorotic area was observed at the base of the leaves when the petiole started to decay. Leaves attached to the plant also retained their green color to an advanced stage of maturity and on through desiccation to eventual decay (Figure 4.7 B). In attached leaves of the yellowing cultivar, chlorosis developed over the entire leaf (figure 4.7 C) in contrast to the veinal pattern of chlorosis observed in previous experiments (Chapter 3).

In the yellowing cultivar, total soluble protein in attached leaves declined during the 12-day period in the dark in the same fashion as in detached ones (Figure 4.8).

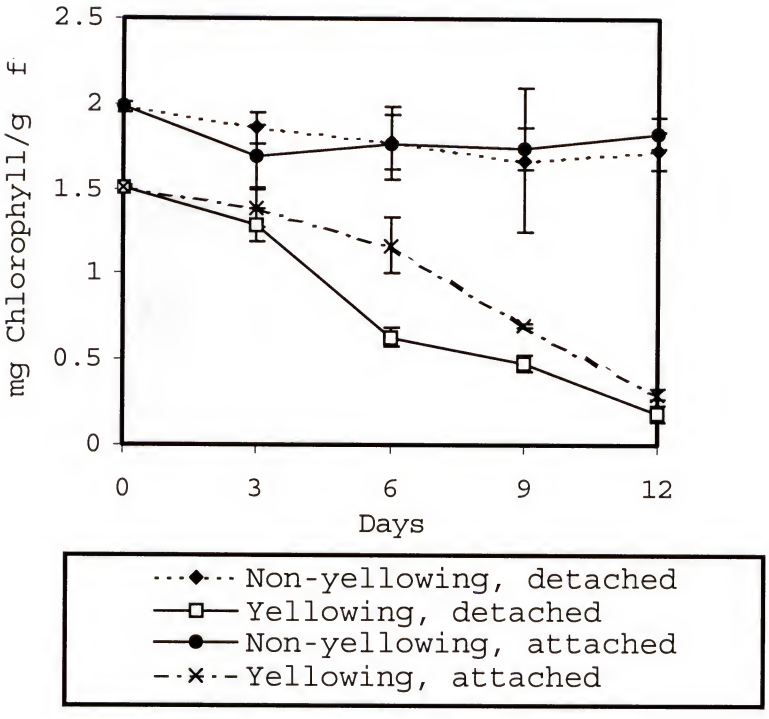

Figure 4.6. Rates of Chl degradation in detached and attached leaves of a yellowing cultivar (Tara) and a non-yellowing cultivar (Boaldi) during dark-induced senescence. Each point represents the mean ± SD of three replicates.

In the non-yellowing cultivar, protein levels in attached leaves are maintained at the initial levels as occurred in detached leaves (Figure 4.9). Protein banding patterns in SDS-polyacrylamide gel electrophoresis for detached and attached leaves of the yellowing cultivar during dark-induced senescence were similar (Figure 4.9 A): in both types of leaves there was a gradual degradation of all the protein bands. In the non-yellowing cultivar, the intensity of the bands were maintained in the two types of leaves (Figure 4.9 B).

Figure 4.7. A) On the left Tara potted chrysanthemum, a yellowing cultivar and Boaldi a non-yellowing cultivar after 12 days in the dark. B) Dry leaves in Boaldi after 18 days in the dark. C) Pattern of leaf chlorosis in attached Tara leaves.

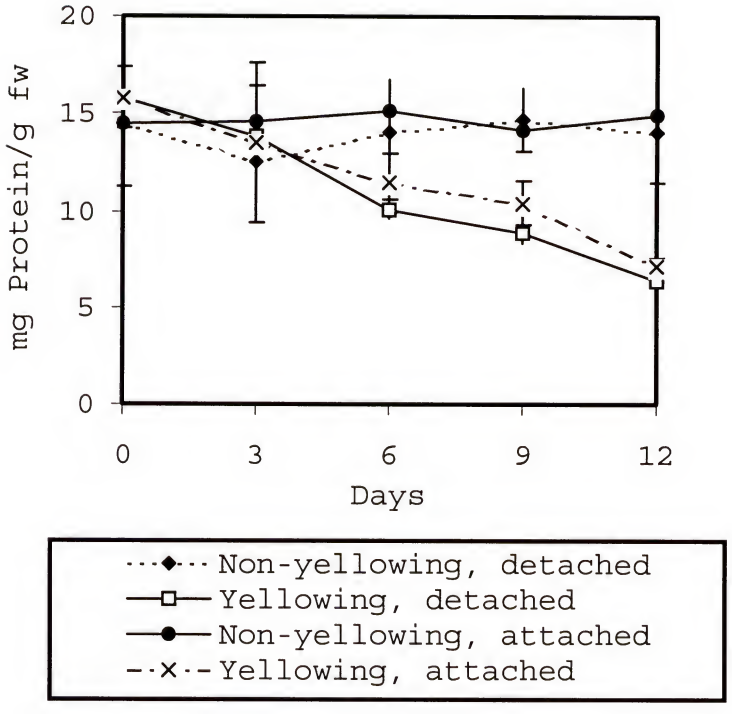

Figure 4.8. Total soluble protein in detached and attached leaves of a non-yellowing cultivar Boaldi, and a yellowing cultivar Tara during dark-induced senescence. Each point represents the mean ± SD of three replicates

The rate of CO_2 evolution in attached and detached leaves of both cultivars showed a steep initial decline after 3 days in the dark (Figure 4.10). The non-yellowing cultivar maintained low rates of CO_2 evolution during the 12-day period. In contrast, in the yellowing cultivar an increase occurred in attached leaves from day 6, and to a lesser extent in detached leaves (Figure 4.10). Detached leaves of the yellowing cultivar were not suitable for measurement at day 12 because the petiole started to decay.

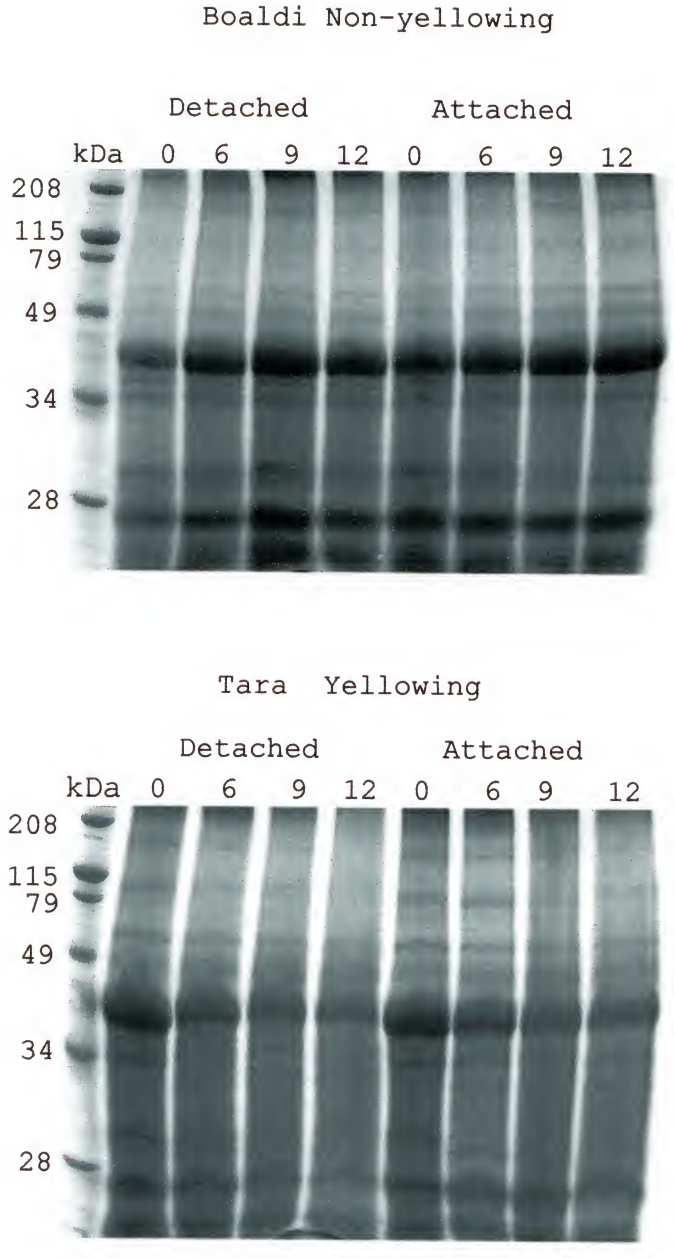

Figure 4.9. SDS-Polyacrylamide gel electrophoresis of total soluble protein in attached and detached leaves of a non-yellowing cultivar Boaldi (top) and a yellowing cultivar Tara (bottom) during dark-induced senescence. Numbers on the top of each line represents days in the dark. Molecular weight markers (kD) are specified in the left line. Each line was loaded with 40 μg of protein.

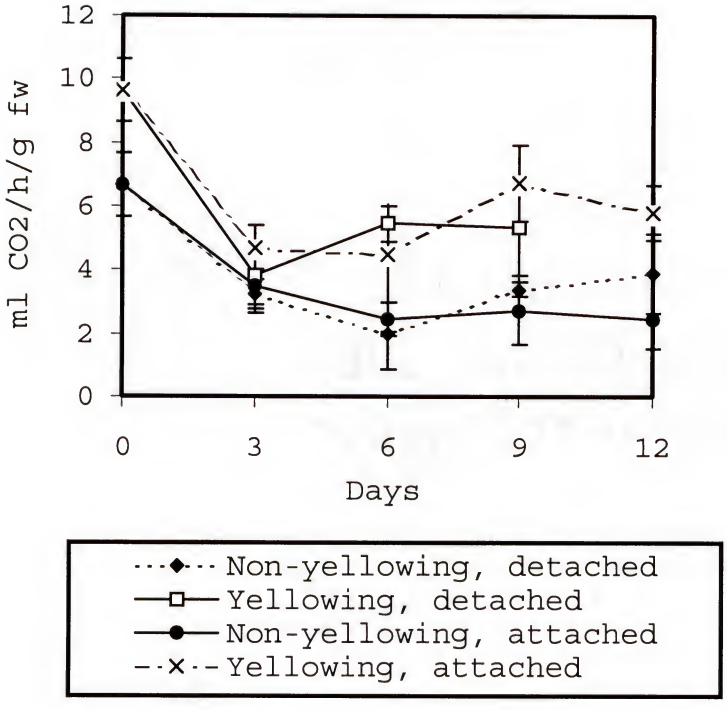

Figure 4.10. Rates of CO_2 evolution in detached and attached leaves of a non-yellowing cultivar Boaldi, and a yellowing cultivar Tara during dark-induced senescence. Each point represents the mean ± SD of three replicates.

Exogenous ethylene, sucrose and benzyladenine

Figure 4.11 shows that ethylene ($100\mu l \cdot l^{-1}$) accelerated the loss of Chl in detached leaves of the yellowing cultivar after 3 days of treatment and that the non-yellowing cultivar was insensitive to this treatment. The experiment was repeated twice with similar results. When samples were taken after 3 days of treatment, chlorosis was not evident in the yellowing cultivar. However, one day after the leaves were taken out of the Plexiglas chamber all the leaves wilted (petioles were still in water) and

chlorosis was apparent in ethylene-treated leaves, while air-exposed leaves did wilt but were not chlorotic.

The decrease in Chl from day 3 to 9 in leaves of the yellowing cultivar was reduced by a 1% sucrose solution. Decreasing the pH in dH_20 water did not affect appearance of chlorosis (data not shown). Sucrose did not affect the non-yellowing cultivar leaves (Figure 4.12).

Benzyladenine (BA) applied to attached leaves at 50 $mg \cdot l^{-1}$ also delayed the loss of chlorophyll in the yellowing cultivar (Figure 4.13). The lower rates of BA tested: 5 and 25 $mg \cdot l^{-1}$ were not different from the control (data not shown).

Discussion

Dark-induced senescence of detached leaves in the yellowing cultivar Tara exhibited a coordination between the different components of the senescence syndrom, i.e. Chl and soluble protein declined and electrolyte leakage increased. The decrease in protein concentration was associated with an increase in proteolytic activity, especially after 9 and 12 days in the dark. This is a classical pattern of senescence described in numerous species (Lohman, 1994; Noodén, 1988a; Roberts et al., 1987; Thimann, 1980). Detached leaves of the non-yellowing cultivar

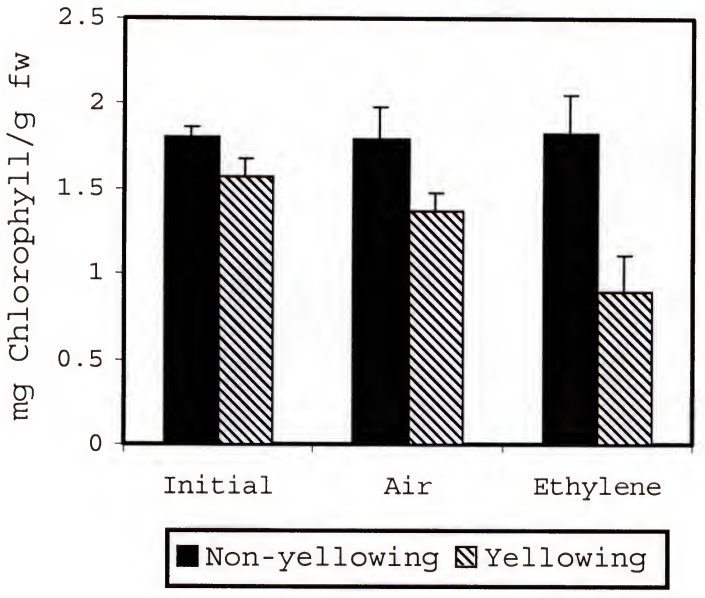

Figure 4.11. Chlorophyll concentration in detached leaves of a non-yellowing cultivar Boaldi and a yellowing cultivar Tara subjected for 3 days to ethylene ($100\mu l \cdot l^{-1}$) or air in the dark. Each point represents the mean ± SD of three replicates.

Boaldi retained high Chl levels and soluble protein concentration during the 12-day period, and the low levels of electrolyte leakage indicated that membrane integrity was maintained.

Delayed or inoperative senescence has been described in a wide number of species and reviewed by Thomas and Smart (1993), who emphasized that the stay-green character may arise from quite different physiological and biochemical modifications. In *Festuca pratensis* L. (Thomas and Stoddart, 1975) and *Phaseolus vulgaris* L. (Ronning et al., 1991), the best studied examples, the non-yellowing mutant genotypes are characterized by a virtually complete disabling of the leaf yellowing processes so that plants remained green throughout the life cycle or when exposed to

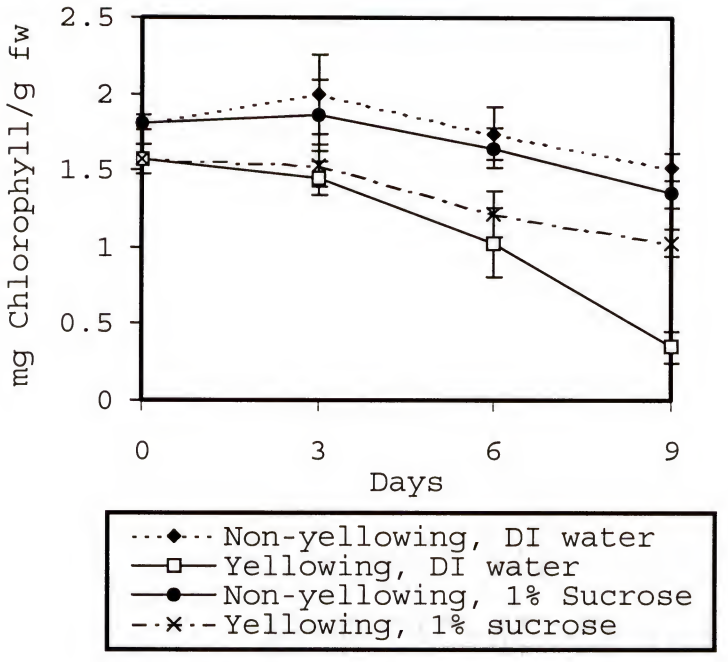

Figure 4.12. Chlorophyll concentration in detached leaves of a non-yellowing cultivar Boaldi and a yellowing cultivar Tara during dark-induced senescence in a 1% sucrose solution or deionized (DI) water. Each point represents the mean ± SD of three replicates.

stresses that will normally cause chlorosis or necrosis. However, other aspects of senescence, such as the decline in soluble protein proceeded as in the yellowing genotype. A more detailed study of the structure of the chloroplast membrane during senescence showed that the major thylakoid polypeptides associated with chlorophyll-protein complexes, CPI and CPII, normally degraded at the same time as chlorophyll in the yellowing cultivar, are retained in the non-yellowing mutant of Festuca (Thomas, 1982a). However, the pattern of proteinases in the non-yellowing *Festuca* mutant was found to be identical to that of the

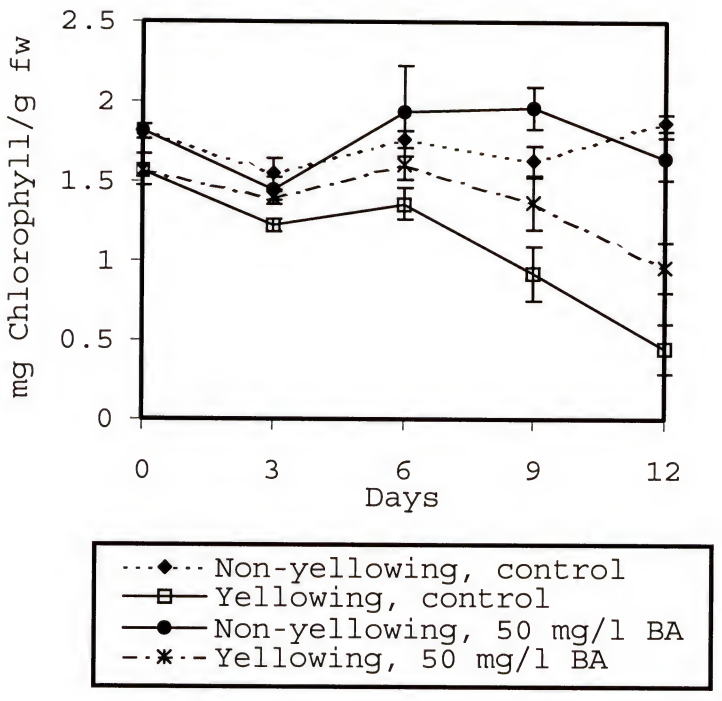

Figure 4.13. Chlorophyll concentration in attached leaves of a non-yellowing cultivar Boaldi and a yellowing cultivar Tara treated with 50 $mg \cdot l^{-1}$ BA or water during dark-induced senescence. Each point represents the mean ± SD of three replicates.

normal genotype (Thomas 1982b). The author suggested that it is the accessibility of thylakoids proteins to proteinase action rather than proteinase per se that is impaired in the non-yellowing mutant. Thomas and Smart (1993) classified stay-green mutants that retain Chl but lack photosynthetic competence as 'non-functional stay-green', which may arise by alteration of genes which regulate chlorophyll catabolism.

Stay-green genotypes that delay the loss of both Chl and photosynthetic activity have been described in cowpea (Gwathmey, 1992), wheat (Wittenbach, 1977), sorghum (Ambler et al., 1987), rice (Soejima et al. 1995) and tobacco (Colbert and Beever, 1981).

Thomas and Smart (1993) defined these as 'functional stay-green'. This pattern may arise after alteration of genes involved either in the timing of the initiation of senescence or in its rate of progress. The non-yellowing chrysanthemum cultivar Boaldi retained both Chl and soluble protein. Photosynthetic activity was not measured in this experiment to classify Boaldi as a 'functional' or 'non functional' stay-green genotype.

Detachment and darkness promotes senescence processes and has been used in the study of leaf senescence (Thomas and Stoddart, 1980). However, these treatments seem to cause a sudden switch from anabolism to catabolism, which is quite different from the subtle shift in metabolism over days or weeks in attached leaves. In fact it has been shown that during dark incubation of detached leaves only a minor part of the mRNA changes observed are related to senescence, whereas stress-related mRNAs predominated quantitatively (Becker and Apel, 1993). Nevertheless, model systems are still justified in certain circumstances, such as comparisons of varieties differing in their pattern of senescence subjected to the same treatment (Smart, 1994). Moreover, the objective of this study is not concerned with natural senescence but with the response of chrysanthemum plants to prolonged dark storage.

Excised leaves, however, display some very substantial differences in their senescence compared with attached ones (Noodén, 1988a). Sinks for the export of breakdown products are

removed and the translocation of hormones from the roots can not occur. To address this problem, dark-induced senescence was compared in detached and attached leaves of both chrysanthemum cultivars.

When whole plants were placed in darkness, the rate of Chl destruction and proteolysis in the yellowing cultivar were closely comparable to the rates in the detached leaves, though Chl destruction was slightly slower. Protein banding patterns in SDS-PAGE were also alike. Similar results were found with oat leaves (Thimann et al., 1974). They reported that proteolysis in the leaf, whether attached or detached, is accompanied by markedly polar basipetal transport of amino acids. The amino nitrogen level in the attached leaves, however, decreases rapidly as amino acids are transported out of the leaf, while in detached leaves it continues to rise as the proteolysis continues.

Respiration rates in detached and attached leaves of the yellowing cultivar followed a typical senescence pattern. The rate of respiration declined after detachment, and increased again when the color of the leaves began to change from green to yellow (Solomos, 1988). A climateric-like rise in respiration has been observed during senescence of detached tobacco, oat, barley and ivy leaves (Solomos, 1988; Tetley and Thimann, 1974; Malik and Thimann, 1980). The respiratory rise, however, was less marked in detached leaves, which differs from the results reported by Thimann et al. (1974) for oat leaves. In fact, if the rise in

respiration is due in part to the increase of respirable substrates in the form of amino acids, then a greater rise should be expected in detached leaves due to the accumulation of these substrates in the leaves.

Attachment made no difference in the non-yellowing cultivar. Chlorophyll and protein concentration remained at high levels as in the detached leaves, and the protein banding patterns in SDS-PAGE were similar. The rate of respiration in detached and attached leaves declined sharply after 3 days in the dark and a low rate was maintained during the 12- day period.

Respiration rates have not been reported in most of the stay-green cultivars described. Ronning et al. (1991) reported similar trends in the rate of respiration of the yellowing and non-yellowing *Phaseolus vulgaris*. However, the non-yellowing *Phaseolus* is a non-functional stay-green and, except for the degradation of Chl, leaf senescence proceeds as in the yellowing cultivar.

This study found that dark-induced senescence in detached and attached leaves of both chrysanthemum cultivars was very similar. This indicates that the results observed with detached leaves were not caused by detachment. It also suggests that a detached leaf is a good model system to study postproduction leaf quality in potted chrysanthemum under prolonged dark storage conditions.

Exogenous ethylene accelerated the decline in Chl in detached leaves of the yellowing cultivar Tara, and chlorosis was visible one day after the end of the treatment (3 days at 100 $\mu l \cdot l^{-1}$). However, ethylene had no effect on the non-yellowing cultivar. Sensitivity of chrysanthemum leaves to exogenous ethylene has not been described previously. Woltering (1987) reported a lack of response of chrysanthemum leaves treated with 0-15 $\mu l \cdot l^{-1}$ ethylene. The concentration of ethylene used in this experiment, 100 $\mu l \cdot l^{-1}$, is frequently used in studies of leaf senescence (Abeles el al., 1988; Grbić and Bleecker, 1995).

Exogenous ethylene promotes leaf senescence processes (Abeles et al., 1988; Mattoo and Aharoni, 1988), and endogenous ethylene is known to be a key hormone in the onset of leaf senescence (Gepstein and Thimann, 1981; Zacarias and Reid, 1990). In many of these studies, Chl content was used as a marker of leaf senescence. Davies and Grierson (1989) isolated cDNAs that accumulate during both leaf senescence and fruit ripening in tomato. Induction of these cDNAs coincided with the peak of ethylene synthesis in both leaves and fruits, and STS, an inhibitor of ethylene action, substantially reduced the expression of marker genes. These results implied that ethylene controls gene expression during leaf senescence as it does during tomato fruit ripening (Davies and Grierson, 1989).

Recently, identification of genetic variants impaired in ethylene production or perception has allowed a more precise

analysis of ethylene function in leaf senescence. Delayed leaf senescence in an ethylene-insensitive mutant of *Arabidopsis*, *etr1-1*, coincides with delayed induction of senescence-associated genes (SAGs) and higher expression levels of photosynthetic-associated genes (PAGs) (Grbić and Bleecker, 1995). In wild type plants, exogenous ethylene was able to further accelerate induction of SAGs and decrease expression of PAGs. However, it was observed that the extended period of leaf longevity in *etr1-1* was associated with low levels of photosynthetic activity. Therefore, the leaves of *etr1-1* functionally senesced even though the apparent life of the leaf was prolonged (Grbić and Bleecker, 1995).

Sucrose had a considerable effect in preserving Chl in the yellowing cultivar Tara (Figure 4.12). Carbohydrate deprivation commonly occurs in higher plants during senescence (Peoples and Dalling, 1988; Tetley and Thimann, 1974), in darkness (Elamrani et al., 1994; Peeters and Van Laere, 1992; Postius and Jacobi, 1971), and under postharvest conditions (King et al., 1990). These studies have shown that cells modify their metabolism to survive in the abscence of carbohydrates by increasing proteolytic activity and fatty acid degradation (Brouquisse et al., 1991). Exogenous sugar solutions preserved Chl loss in oat leaves segments (Tetley and Thimann, 1974) and reversed starvation of maize root tips (Brouquisse, 1991).

Benzyladenine (50 $mg \cdot l^{-1}$) applied one day before the plants were placed in the dark also reduced the development of leaf chlorosis in the yellowing cultivar. The role of cytokinins in delaying senescence of detached leaves and leaf disks or segments has been confirmed in numerous studies (Mok, 1994; Sabater et al. 1990a; Van Staden et al., 1988). Even though intact plants are generally less responsive to cytokinins, probably due to correlative influences of other plant parts, cytokinins also delay senescence of attached leaves. Benzyladenine (50-100 $mg.l^{-1}$) reduced leaf chlorosis in potted miniature roses (Clark et al., 1991; Tjosvold et al., 1994) and in Dieffenbachia (Ben-Jacoov et al., 1985).

Recently, the ability to transform plants with foreign genes has made it possible to alter the endogenous cytokinin level. With the availability of more genes, like *tmr* involved in cytokinin biosynthesis (Smart, 1991), the link between cytokinin concentration and plant development will be strengthened.

In summary, dark-induced leaf senescence in Tara, whether detached or attached followed a typical pattern of senescence. Boaldi leaves, on the other hand, did not degrade clorophyll or soluble protein during the 12 days in the dark. These observations suggest that Boaldi is a type of stay-green genotype, most probably a 'functional type', in which genes involved in the initiation of senescence have been altered.

These types of stay-green phenotype are of potential importance in agronomic crops since they continue to photosynthesize for longer than normal, and might be expected to show a higher yield in crops for which carbohydrate is a major component of the harvest. In ornamental crops, where extended greenness is a highly desirable characteristic, this type of stay-green phenotype will have great relevance in the future.

CHAPTER 5
SUMMARY AND CONCLUSIONS

Premature leaf yellowing in some potted chrysanthemum cultivars has been a problem reported by growers under different cultural and shipping conditions (Carl Scharfenberg, Yoder Brothers, Inc., personal communication). This study was conducted to 1) elucidate the production and storage conditions that can induce leaf yellowing, 2) characterize leaf yellowing symptoms and 3) investigate the senescence pattern of two chrysanthemum cultivars, Tara a yellowing cultivar and Boaldi a non-yellowing cultivar.

Of the factors evaluated, cultivar selection had the greatest influence on chrysanthemum leaf yellowing. Boaldi leaves never showed chlorosis regardless of the treatments imposed. Tara, on the other hand, was susceptible to a variety of production and storage conditions and often the interaction of both triggered leaf yellowing. Thus, two different problems were observed and studied: One was the production and shipping conditions that negatively affected postproduction leaf quality in Tara, and the other was differences between the two cultivars in the rate or pattern of senescence.

At least three different leaf injury symptoms were observe in Tara, which eventually resulted in premature leaf senescence. First, dryness and necrosis of bottom leaves, and marginal necrosis and chlorosis of upper leaves were associated with high fertilizer levels. Under prolonged dark storage conditions (21°C) chlorosis and wilting eventually affected all leaves. Heavy fertilization stimulates costly processes for ion compartmentalization (Penning de Vries, 1975), and prolonged darkness induces carbohydrate starvation (Krapf and Jacobi, 1975). Leaf chlorosis in Easter lilies (Jiao et al., 1986; Miller et al, 1995) and leaf blackening in *Protea* (Bieliski et al. 1992; Dai and Paull, 1995; McConchie and Lang, 1993) have been related to reduced stored carbohydrates. Probably, high nutritional levels together with prolonged dark storage induced depletion of intracellular carbohydrates and resulted in premature leaf senescence in Tara.

The second set of symptoms were similar to chilling injury i.e. loss of turgor, wilting and chlorosis and were observed in Tara after storage at 12°C when the experiments were conducted in late spring and early fall. Susceptibility to 12°C was not observed in winter experiments. However, the fact that these symptoms did not appear when the plants were stored at 4°C suggests that chilling injury was probably not the cause of leaf injury.

Veinal leaf chlorosis was the third symptom and was characteristic of multiflowered plants, while disbudded plants did not show any chlorosis (stored for 4 days at 21°C). Disbudding and decapitation delay leaf senescence of the remaining leaves and increases the cytokinin activity in these leaves (Van Stadden et al., 1990). A decrease in cytokinin production in the roots could be involved in inducing leaf chlorosis in multiflowered Tara.

All these symptoms were observed in experiments conducted either in late spring or early fall. No major chlorosis or susceptibility to 12°C was observed in the winter experiments. These results may explain why growers reported problems with leaf yellowing under different cultural, season and transport conditions.

Dark-induced senescence studies showed that chlorophyll and soluble protein concentration declined and electrolyte leakage increased in detached Tara leaves over a 12-day period. The decrease in protein concentration was accompanied by an increase in proteolytic activity following a typical pattern of senescence (Thomas and Stodart, 1980). Detached leaves of Boaldi retained high chlorophyll and soluble protein concentrations during the same period in the dark and did not show any significant increase in proteolytic activity. These results suggest that Boaldi is a 'stay-green' or non-yellowing cultivar, in which genes involved in the initiation of senescence have been altered. Delay or

inoperative senescence has been described in a wide number of species (Thomas and Smart, 1993). These types of stay-green phenotypes are of a great potential importance in the ornamental industry where extended greenness is a highly desirable characteristic.

APPENDIX A

Nutrients levels, soluble sugars and starch concentration in leaf tissue at the end of production from Experiment 1 in 1995 are shown in Table A-1 and A-2, and A-3.

Table A-1. Effect of fertilizer and cultivar on leaf K, Zn, Fe, Cu and Na. (Experiment 1, 1995).

	K (%)	Zn ($mg \cdot l^{-1}$)	Fe ($mg \cdot l^{-1}$)	Cu ($mg \cdot l^{-1}$)	Na ($mg \cdot l^{-1}$)
Fertilizer					
Low	6.8	33.8	86.1	8.2	364.2
Medium	8.3	45.3	104.9	14.0	347.5
High	8.1	39.1	143.4	14.0	320.0
Linear[z]	0.022		0.006	0.006	
Quadratic[y]	0.013		0.986	0.015	
Pr>F	0.001	0.339	0.035	0.005	0.019
Cultivar					
Boaldi	7.2	30.2	112.2	12.5	281.7
Tara	8.3	48.7	110.7	11.7	406.1
Pr>F	0.001	0.019	0.924	0.525	0.001

[z],[y] P value for the linear and quadratic orthogonal contrast respectively.

Table A-2. Interaction of fertilizer (F) and cultivar (C) on leaf N, P, Ca, Mg, Mn, and B. (Experiment 1, 1995).

		N (%)	P (%)	Ca (%)	Mg (%)	Mn ($mg \cdot l^{-1}$)	B ($mg \cdot l^{-1}$)
Cultivar	Fertilizer						
Boaldi	Low	4.5	0.74	1.10	0.43	185	38.3
	Medium	5.6	0.76	1.13	0.48	225	58.0
	High	6.7	1.01	0.84	0.41	208	79.8
Linear[z]		0.001	0.008	0.001	0.107	0.242	0.001
Quadratic[y]		0.001	0.362	0.025	0.007	0.022	0.043
Tara	Low	3.9	1.04	1.06	0.42	144	61.8
	Medium	5.2	2.01	1.37	0.69	172	72.8
	High	5.6	2.65	1.19	0.67	227	84.2
Linear[z]		0.001	0.001	0.157	0.001	0.001	0.001
Quadratic[y]		0.001	0.001	0.001	0.001	0.971	0.189
F x C[x]		0.002	0.001	0.001	0.001	0.008	0.003

[z],[y] P value for the linear and quadratic orthogonal contrast, respectively.
[x], P value for the fertilizer and cultivar interaction.[x]

Table A-3. Effect of fertilizer (F) and cultivar (C) on leaf soluble sugars and starch concentration at flowering (expressed in mg glucose $g.dw^{-1}$ (Experiment 1, 1995).

	Soluble sugars	Starch
Fertilizer(F)		
Low	25.5	28.8
Medium	17.4	14.6
Low	14.9	9.4
Pr>F	0.001	0.001
Linear[z]	0.001	0.001
Quadratic[y]	0.002	0.086
Cultivar		
Boaldi	23.4	26.0
Tara	15.2	9.2
Pr>F	0.001	0.001
F X C	0.5695	

[z],[y] P value for the linear and quadratic orthogonal contrast, respectively.

APPENDIX B

Introduction

This experiment was conducted as part of this project during the winter months to identify the combination of temperature and duration during simulated shipping that may induce premature chlorosis in Tara.

Materials and Methods

Tara rooted cuttings were planted on November 11, 1995. Plants received (in $mg \cdot l^{-1}$) 300 N, 72 P and 249 mg K from 20N-4.7P-16.6K at each watering. Three blocks or replicates were imposed across the temperature gradient. Average maximum irradiance levels at noon were 471 $\mu mol \cdot m^{-1} \cdot s^{-2}$, and average maximum day and minimum night temperatures were 25.2 and 17.2°C, respectively. At flowering, on January 8, two plants per replicate were placed in plastic sleeves, boxed and stored in the dark at 12, 21 or 29°C for 2, 4 or 6 days. The plants were then placed in an interior holding room held at $20 \mu mol \cdot m^{-2} \cdot s^{-1}$ of PPF from cool-white fluorescent lamps, at 21±1 °C for 12 hours, and

50±5% RH. Visual leaf quality was rated two weeks after flowering from poor to excellent as follows: 1=plants with ≥ 50% chlorotic or necrotic leaves; 2=plants with ≤ 50% and ≥ 25% chlorotic or necrotic leaves; 3=plants with ≤ 25% and ≥ 15% chlorotic or necrotic leaves; 4=plants with ≤ 15% chlorotic or necrotic leaves; and 5=no chlorotic or necrotic leaf margins. The data were analyzed using analysis of variance procedure and orthogonal comparisons were used to examine differences among treatment means.

Results and Discussion

Storage duration did not affect plants stored at 12 and 21°C, but increasing storage from 4 to 6 days at 29°C slightly reduced leaf quality (Table B-1). These results correspond to those reported by Nell et al. (1989). No major chlorosis developed in this experiment. The lower quality rating was mostly due to marginal necrosis. The susceptibility (loss of turgor and wilting) of Tara to storage at 12°C in experiments conducted in late spring was not observed in this experiment.

Table B-1. Interaction of temperature (T) and dark storage duration (D) on postproduction leaf quality of Tara.

Temperature (°C)	Storage Duration (days)	Leaf Quality rating[z]
12	2	4.3
	4	4.1
	6	4.2
Linear[y]		0.128
Quadratic[x]		0.340
21	2	4.1
	4	4.3
	6	4.3
Linear[y]		0.327
Quadratic[x]		0.684
29	2	4.4
	4	4.3
	6	3.6
Linear[y]		0.031
Quadratic[x]		0.864
T x D		0.041

[z],Quality rating was evaluated 2 weeks after flowering,
1= severely damaged.
2= unacceptable quality, no ornamental value.
3= visible leaf damage, but still acceptable for the consumer.
4= no longer a perfect product, but had no evident damage.
5= no damaged leaves.
[y],[x] P value for the linear and quadratic orthogonal contrast, respectively

APPENDIX C

Introduction

Research conducted on Torch and Spirit chrysanthemum cultivars (Carver et al., 1993) and other crops (ter Her and Hendriks, 1995) reported a detrimental effect of high nitrogen levels on postproduction plant quality. This experiment was also conducted as part of this project to evaluate the effect of nitrogen rate on Tara postproduction leaf quality.

Materials and Methods

Tara chrysanthemum rooted cuttings were planted in 11.25-cm (640 cm^3) plastic pots using commercial potting media (Vergro Klay Mix, Verlite Co. Tampa, Fl.). Cultural practices were the same as specified in Chapter 1, Experiment 1. Fertilizer treatments included three nitrogen rates (75, 150 and 300 mg $N \cdot l^{-1}$) using ammonium nitrate. Others nutrients in each solution were kept constant at (in $mg \cdot l^{-1}$) 20 P, 180 K, 80 Ca, and 90 Mg from phosphoric acid, potasium sulfate, calcium sulfate and magnesium sulfate, respectively. Electrical conductivity of the fertilizer solutions was 1.9, 2.2 and 2.9, respectively.

Nitrogen treatments were applied at every irrigation throughout production. Plants were watered as needed with fertilizer solution (250 ml) and leachate collected was recorded. The experiment was repeated twice. Plants were water 13 times during the winter experiment and 16 times during the spring experiment. Planting dates, flowering dates, average maximum irradiance, average maximum and minimun temperatures and total N applied are shown in table C-1. Total N applied was calculated by substracting the leached volume per pot from the total volume applied (250ml/pot).

Table C-1. Experimental conditions.

Planting date	Flowering date	Max PPFD (μmol $m^{-2}s^{-1}$)	Max/min Temp °C)	Total N (mg)
11 Nov 1995	8 Jan 1996	471	25.0/17.2	112 (75 $mg \cdot l^{-1}$) 292 (150 ") 631 (300 ")
7 March 1996	13 May 1996	655	27.5/17.4	172 (75 $mg \cdot l^{-1}$) 360 (150 ") 780 (300 ")

Greenhouse experiments were arranged as a randomized complete block design with 3 replications and 4 plants per experimental unit.

At flowering, two plants per treatment per replicate were used to determine soil media electrical conductivity according to Wright (1986), and the leaf nutrient level. Total leaf nitrogen was analyzed following Hanlon et al. (1994) procedures. For

phosphorus, potassium, calcium, magnesium, manganese, copper, iron, zinc, sodium and boron analysis, 1.0 g of oven-dry plant tissue was ashed at 500° C for 5 hours and digested with 5 ml of 6.0M HCl. After filtering, elements in the digests were determined using inductively coupled plasma spectroscopy (ICAP 61-E, Thermo Jarrell-Ash Corp., Franklin, MA).

Plants were stored at 21°C for 4 days. After storage, the plants were placed in a interior holding room held at 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of PPFD from cool-white fluorescent lamps for 12 hours/day. Temperature was 21±1°C and relative humidity (RH) 50±5%. Visual quality was rated two weeks after flowering from poor to excellent as follows: 1=plants with ≥ 50% chlorotic or necrotic leaves; 2=plants with ≤ 50% and ≥ 25% chlorotic or necrotic leaves; 3=plants with ≤ 25% and ≥ 15% chlorotic or necrotic leaves; 4=plants with ≤ 15% chlorotic or necrotic leaves; and 5=no chlorotic or necrotic leaf margins.

Since plants in spring received more nitrogen due to more frequent irrigation, and more damage was observed, the data were analyzed as a split-plot over time with 'season' as a main plot. The data were analyzed using analysis of variance procedure, and orthogonal comparisons were used to examine differences between treatment means.

Results and Discussion

No major leaf chlorosis or wilting occurred in this experiment. In the winter experiment, postproduction leaf damage was minimal and only small dry marginal areas were observed; increasing N rates did not affect postproduction leaf quality in winter, and in spring leaf quality slightly decreased as nitrogen rates increased from 150 to 300 mg $N \cdot l^{-1}$ (Table C-2). Leaf damage during postharvest was characterized by marginal browning and dryness.

In both seasons, increasing the nitrogen rates significantly increased media soluble salts (Table C-2). However, this increase was not accompanied by a notable difference in the postproduction leaf quality in the winter, and only a slight decrease in leaf quality occurred in the spring experiment. Media soluble salts were higher in spring than in winter. During the spring experiment, due to higher temperature and irradiance plants received more fertilizer solution and more nutrients than in winter, which resulted in a small but significantly higher EC (Table C-2).

Increasing fertilizer rate increased leaf N, K, and Mg in both seasons and leaf Mn in spring but not in winter. Leaf N, K, Ca and Na were higher in spring than in winter (Table C-3).

The results of this experiment seem to indicate that high nitrogen rates or elevated soluble salts in the soil media are not the cause of premature leaf yellowing in Tara. However, the highest nitrogen rate applied in spring slightly but significantly decreased leaf quality in Tara. Given the seasonal interactions observed in other experiments, it can not be concluded that high nitrogen rates do not induce premature leaf senescence in Tara.

Table C-2. Media electrical conductivity (EC) at flowering and leaf quality rating after dark storage at 21°C C for 4 days.

	EC ($dS \cdot m^{-1}$)	Quality rating[z]	
		Season	
Fertilizer (F) ($mg\ N \cdot l^{-1}$)		Winter	Spring
75	3.4	3.8	4.3
150	3.9	4.2	3.8
300	6.7	4.3	3.4
Pr>F	0.001		
Linear[z]	0.001	0.118	0.017
Quadratic[y]	0.001	0.284	0.640
Season(S)			
Winter	4.4		
Spring	4.9		
Pr>F	0.001		
F X S	0.325	0.013	

[z]Quality rating was evaluated two weeks after flowering, 1=poor; 5=excellent quality.

[y],[x] P value for the linear and quadratic orthogonal contrast, respectively.

Table C-3. Effect of nitrogen rate on leaf N, K, Ca, Mg, Na and Mn concentration.

	N(%)	K(%)	Ca(%)	Mg(%)	Na ($mg \cdot l^{-1}$)	Mn ($mg \cdot l^{-1}$)	
						Winter	Spring
Fertilizer (F) ($mg\ N \cdot l^{-1}$)							
75	4.2	7.2	1.2	0.44	275	168	213
150	4.9	8.7	1.25	0.53	270	159	235
300	5.5	8.2	1.29	0.55	308	173	272
Pr>F	0.001	0.001	0.001	0.001	0.262		
Linear[z]	0.001	0.134	0.191	0.001		0.394	0.001
Quadratic[y]	0.135	0.012	0.685	0.003		0.141	0.514
Season (S)							
Winter	4.4	7.3	1.11	0.48	257		
Spring	5.3	8.8	1.33	0.53	311		
Pr>F	0.022	0.012	0.002	0.050	0.003		
F x S	0.472	0.073	0.129	0.227	0.878	0.001	

[z],[y] P value for the linear and quadratic orthogonal contrast, respectively

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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